UNIVERSITY OF ALBERTA

THE ROLE OF HYPERBARIC OXYGEN IN SPORTS MEDICINE

by

Anthony Lindsay Webster

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of Doctor of Philosophy

Faculty of Physical Education and Recreation

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled "The Role of Hyperbaric Oxygen in Sports Medicine" submitted by Anthony Lindsay Webster in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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ABSTRACT

In the last decade, interest has emerged in hyperbaric oxygen therapy (HBO) in the field of sports medicine. Elite athletes have undergone HBO in the belief that it may be an ergogenic aid for subsequent physical performance and also may accelerate recovery from sports injuries. Unfortunately, there has been very little scientific research to support the efficacy of HBO for these purposes.

The purpose of this thesis was to investigate the role of hyperbaric oxygen therapy (HBO) in sports medicine. Two studies were performed. Study 1 was designed to determine if administration of HBO prior to an incremental exercise test to exhaustion had the ability to improve or alter performance during that exercise test. Study 2 was designed to determine if administration of three HBO treatments had the ability to accelerate recovery from exercise-induced muscle damage within human gastrocnemius muscle.

In study 1, no difference was found in maximal oxygen consumption, ventilation threshold or lactate threshold during exercise after HBO relative to baseline exercise tests without prior HBO. Muscle oxygenation during exercise after HBO was improved only at one power output of the exercise test. Therefore, prior exposure to HBO had no demonstrable ergogenic effect on subsequent incremental exercise performance.

In study 2, recovery from exercise-induced muscle damage was monitored using isokinetic dynamometry, magnetic resonance imaging and spectroscopy of the lower leg and also measures of pain sensation and unpleasantness. The rate of recovery was not different in the HBO and control groups for all variables except isometric peak torque

and pain sensation and unpleasantness. In these cases, recovery was more rapid in the HBO group. This study failed to provide convincing evidence that HBO was capable of accelerating overall recovery from exercise-induced muscle damage.

In summary, the research performed in this thesis failed to demonstrate that HBO has a significant role to play in sports medicine either as an ergogenic aid prior to performance or as a therapeutic modality for acceleration of recovery from exercise-induced muscle damage.

Key Words: Hyperbaric Oxygen Therapy; Sports Medicine; Ergogenesis; Athletic Injuries.

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LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
ATA	Atmospheres absolute.
CSA	Cross-sectional area
DDS	Descriptor Differential Scale
DOMS	Delayed-onset muscle soreness
EIMD	Exercise-induced muscle damage
HBO	Hyperbaric oxygen therapy
ISIS	Image selected in-vivo spectroscopy
LT	Lactate threshold
MRI	Magnetic resonance imaging
MRS	Magnetic resonance spectroscopy
NIRS	Near infrared spectroscopy
NMR	Nuclear magnetic resonance
NNLS	Non-negative least squares
RER	Respiratory exchange ratio
SD	Standard deviation
STEAM	Stimulated echo acquisition mode
VO_2	Oxygen consumption
VO_2max	Maximal oxygen consumption
VT	Ventilation threshold
%Mox	Percent muscle oxygenation

CHAPTER ONE

An Introduction

Introduction

In today's society where professional sport commands vast sums of money, there is great pressure on the sports medicine community to restore injured athletes to full activity as quickly as possible. In addition, pressure is placed on the athletes themselves to ensure that they perform to the best of their ability. An unfortunate result of this situation is that therapies, medications and devices are often used before their efficiency has been scientifically validated (American Orthopaedic Society for Sports Medicine, 1998). A typical example in recent years has been the emergence of hyperbaric oxygen (HBO) as an adjunct therapy in sports medicine.

HBO therapy involves inhalation of an oxygen enriched gas mixture (usually 100% oxygen) at a pressure greater than 1 atmosphere absolute (1 ATA, equivalent to 760mmHg) (Grim *et al.*, 1990; Tibbles & Edelsberg, 1996). Under these conditions, the partial pressure of oxygen is greater than can be achieved when breathing 100% oxygen at sea level. The therapy is conducted either in a chamber which is pressurized with ambient air and the patient breathes 100% oxygen through a mask, or in a monoplace chamber in which pressurized 100% oxygen is circulating freely. Hyperbaric oxygen has several clinical indications for which it is an important treatment including decompression sickness, arterial gas embolism, carbon monoxide poisoning, clostridial myonecrosis, exceptional blood loss anaemia, compromised skin grafts and flaps and prevention of osteoradionecrosis (Grim *et al.*, 1990; Tibbles & Edelsberg, 1996).

In recent years, there has been much interest in hyperbaric therapy in the field of sports medicine (James *et al.*, 1993; James, 1993; Potera, 1995). A number of North

American professional sports teams have purchased hyperbaric chambers in the belief that HBO treatment will help athletes recover more quickly from injury and strenuous exercise (Jain, 1990; Staples & Clement, 1996) and also may help to "energize" them for optimal performance (Potera, 1995). However, there is currently little convincing scientific evidence to support any of these claims.

HBO is not without its hazards. There are significant potential complications associated with hyperbaric therapy. These include possible barotrauma as a result of the mechanical effect of increasing pressure and the potentially serious toxic effects of increased concentrations of oxygen (Grim *et al.*, 1990; Tibbles & Edelsberg, 1996). It is quite possible that the risks associated with hyperbaric therapy (especially when administered by unqualified technicians) may outweigh the potential benefits, especially in the sports medicine setting. There is also the issue of cost: an average 90 minute HBO treatment in the United States costs between $300 and $400 (Tibbles & Edelsberg, 1996). It is quite possible that use of HBO for sports medicine purposes may simply amount to a waste of money that could be better spent on other more traditional or effective forms of sports therapy. Therefore, there is a need for research to clarify the role of HBO in sports medicine.

Purpose of the research:

The purpose of the research detailed in this thesis was to clarify two potential roles of HBO in the sports medicine setting. Two separate studies were completed and are outlined briefly below.

Study 1:

Significance of the research:

Despite the fact that there was virtually no supportive scientific evidence, reports began to emerge in the media in the mid 1990's that athletes were undergoing HBO immediately prior to their sport in the belief that their performance would be improved (Potera, 1995). The media reports eventually prompted the Undersea and Hyperbaric Medical Society in 1994 to issue a press release stating that the use of HBO for performance improvement was without scientific basis and that controlled human studies of the role of HBO in sports medicine were needed.

Very few studies have addressed the issue of whether acute HBO has an effect on performance during subsequent exercise in normoxic, normobaric conditions (Banister *et al.*, 1970; Hoffmann *et al.*, 1990; Cabric *et al.*, 1991). Results of these studies have been discrepant with two (Cabric *et al.*, 1991; Banister *et al.*, 1970) suggesting enhanced performance and one (Hoffmann *et al.*, 1990) finding no effect. All studies performed thus far have suffered from a number of methodological limitations.

Clearly the effect of acute HBO on subsequent normobaric, normoxic performance deserved clarification, especially in light of interest in its use in the field of sports medicine and reports of its use for ergogenic purposes. This study was designed to address this issue.

Research objective:

To determine if administration of HBO (100% oxygen at 2.0 ATA for 60 minutes) prior to an incremental exercise test to exhaustion had the ability to improve or alter performance during that exercise test.

Statistical Hypothesis:

Administration of HBO immediately prior to an incremental exercise test to exhaustion would have no performance-enhancing effects.

Study 2:

Significance of the research:

The use of HBO therapy by professional athletes to accelerate recovery from athletic injuries began to gain popularity in the early 1990's (James *et al.*, 1993; James, 1993). Since then, numerous professional sport franchises have purchased hyperbaric chambers in the belief that it will help their athletes recover from injury more rapidly and therefore return to competition sooner. This has occurred despite the fact that there is still no convincing scientific evidence that supports the use of HBO for athletic injury treatment. The reports that have supported the use of HBO in sports medicine have been almost entirely anecdotal and most of the studies performed to date that have discovered a beneficial effect of HBO for soft tissue injuries have been either been subject to

methodological flaws (James *et al.*, 1993; Borromeo *et al.*, 1997) or have not been performed on humans (Webster *et al.*, 1996; Best *et al.*, 1998).

In order to examine the effect of HBO on soft tissue injury recovery, the present research study used lengthening (eccentric) contractions to elicit exercise-induced muscle damage (EIMD) within the gastrocnemius muscle of untrained male subjects. Two previous studies (Staples, 1999; Harrison *et al.*, 1999) have utilised the EIMD model to investigate the effect of HBO on recovery. Staples (1999) determined that there was an enhanced recovery of eccentric strength with HBO treatment but Harrison *et al.* (1999) were unable to detect any effect of HBO on recovery of EIMD.

Clearly, the use of HBO as an adjunctive therapy in sports medicine is still highly controversial and needs clarification. This study was designed to provide scientific information with regards to the efficacy of HBO for enhancement of recovery from muscle damage and pain induced by eccentric exercise.

Research objective:

To determine if administration of HBO (three treatments of 100% oxygen at 2.5 ATA for 60 minutes) had the ability to accelerate recovery from EIMD within human gastrocnemius muscle.

Statistical Hypothesis:

HBO would be ineffective in accelerating the recovery from EIMD.

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CHAPTER TWO

Review of literature.

The review of literature will begin by providing a brief history of hyperbaric oxygen therapy (HBO), followed by a review of the major physiological effects of HBO and the major clinical indications for this therapy. Finally, a summary will be provided of the research performed to date on the role of HBO in sports medicine.

(1) History of Hyperbaric Medicine.

As might be expected, the origins and development of hyperbaric medicine are closely tied to the history of diving medicine (Jain, 1990). The various unpleasant physical consequences of venturing beneath the world's oceans were observed soon after men began diving (as far back as 4500 B.C.) and led ultimately to the many applications of hyperbaric therapy utilized today by modern medicine (Jain, 1990).

The first use of diving equipment to extend the limits of underwater activity has been attributed to Alexander the Great who, in 320 B.C., is said to have been lowered into the Bosphorus Strait in a glass barrel (Jain, 1990). About two thousand years later in 1620 Cornelius Drebbel developed the first true diving bell that became the forerunner of all submersible vehicles. In 1691, Edmund Halley advanced diving bell technology further by devising a system which allowed air replenishment and the next two centuries saw the development of compressed air diving helmets and suits which allowed extended stays underwater (Jain, 1990).

Despite the fact that dive durations had been extended, they were still limited to

shallow water (Jain, 1990). Divers were still dogged with depth problems such as eardrum rupture and, in particular, decompression sickness ("the bends"). The effectiveness of countering decompression sickness with hyperbaric recompression was finally realised in the mid 19th century by Pol an Watelle of France (Jain, 1990). Soon after, the first hyperbaric chambers were built in Europe and they were soon advertised as being comparable to health spas. Patients with a variety of ailments were treated despite the fact that there was no real rationale for hyperbaric air treatment (Jain, 1990).

The first hyperbaric chamber on the North American continent was constructed in 1860 in Oshawa, Canada just east of Toronto (Jain, 1990). Probably the most famous chambers in history were constructed by Cunningham in the USA in the 1920's. One of these was the largest chamber ever built - five stories high and 64 feet in diameter complete with bedrooms and all the amenities of a hotel. Initially he used his chambers to treat the victims of the Spanish influenza epidemic, his rationale being that mortality from the disease was higher in areas of higher elevation and therefore a barometric factor was involved (Jain, 1990). Despite a tragic accident at one stage where a mechanical failure resulted in sudden loss of compression and the death of many patients, Cunningham continued to promote his treatment as therapy for a number of diseases such as syphilis, hypertension, diabetes mellitus and cancer (Jain, 1990). Eventually, after publicity surrounding his treatments grew, the Americal Medical Association (AMA) began to request Cunningham to document his claims about the efficacy of hyperbaric therapy. Cunningham repeatedly ignored these requests and was subsequently censored by the AMA in a report that stated that Dr. Cunningham appeared to be concerned more

with economics than scientific medicine and that "it is the mark of a scientist that he is ready to make available the evidence on which his claims are based" (Jain, 1990). As a result, the era of hyperbaric air therapy for medical disorders ended.

Initially, largely as a result of the discovery of the toxic effects of concentrated oxygen, there was hesitation to use it under pressure as a form of therapy. However, in 1917, Drager discovered the potential benefits of using oxygen under pressure to treat diving accidents. Benke and Shaw in 1937 were actually the first to administer hyperbaric oxygen for the treatment of decompression sickness and with this the age of hyperbaric oxygen therapy arrived. The time period between 1937 and the present day has seen a surge of interest and information in the area of hyperbaric medicine and now there are numerous North American and international societies concerned solely with the advancement of knowledge in this area.

(2) The Physiological Effects of Hyperbaric Oxygen Therapy (HBO)

There are really only two basic effects of HBO on the human body (Hammarlund, 1994). The first is the mechanical effect of increased pressure which is useful in reducing bubble size within the bloodstream (such as those found during decompression sickness and air or gas embolism). This mechanical effect is governed by Boyle's Law which states that the volume of a gas in an enclosed space is inversely proportional to the pressure exerted on it (Tibbles & Edelsberg, 1996). Second is the biochemical/physiological effect of hyperoxia (ie. increased partial pressures of oxygen in

the tissues of the body) (Hammarlund, 1994). It is the latter effect which is of primary importance in the treatment of most of the clinical conditions for which HBO has been used, including sports injuries. The following is a review of the major physiologic effects of HBO.

(2.1) The physiology of oxygen transport under conditions of HBO

At sea level (ie. 1 atmosphere absolute, ATA), man is exposed to an atmospheric pressure equivalent to 760 mmHg (Fife & Camporesi, 1991). Approximately 160 mmHg of this pressure is exerted by oxygen (21%). After allowance for alveolar water vapour pressure (47 mmHg) and alveolar carbon dioxide tension (40 mmHg in a healthy individual), mean alveolar oxygen tension varies between 100 and 110 mmHg (Fife & Camporesi, 1991). Due to regional ventilation-perfusion inequalities and small venoarterial shunts that are present even in normal individuals, measured arterial partial pressure of oxygen (P_aO_2) in a young healthy individual usually varies from 90 to 100 mmHg (Fife & Camporesi, 1991).

Oxygen (O_2) is transported in blood by two mechanisms: chemical binding to haemoglobin and physical dissolution. Under normal environmental conditions, O_2 is delivered to tissues almost entirely through reversible binding with haemoglobin. As each gram of haemoglobin carries 1.34 ml of O_2 and blood typically contains about 15 grams of haemoglobin per 100 ml blood, this corresponds to a total bound O_2 content of approximately 20 ml per 100 ml blood (ie. 20 vol%) when haemoglobin is fully saturated

(Fife & Camporesi, 1991). In contrast to this, there is an almost inconsequential amount of O_2 physically dissolved in plasma. At a normal P_aO_2 of 100 mmHg, only 0.003 ml of O_2 are dissolved per ml of arterial blood. As a result, only 0.30 ml (0.31%) of O_2 per 100 ml arterial blood are carried in solution at normal P_aO_2 and normal body temperature (Fife & Camporesi, 1991). However, once haemoglobin is fully saturated, further increases in O_2 partial pressure can affect only the plasma dissolved O_2 fraction.

In the range of O_2 partial pressures commonly used in hyperbaric therapy, the amount of physically dissolved O_2 increases dramatically. While breathing 100% O_2 at 3 ATA (2280 mmHg), approximately 6 vol% O_2 can be carried physically dissolved in plasma - an amount sufficient to sustain life in animals in the virtual absence of circulating haemoglobin (Boerema *et al.*, 1960). Thus, HBO has been used with success for transient support of severely anaemic patients when appropriately cross-matched blood products have not been immediately available (Hart, 1994).

(2.2) Haemodynamic and tissue effects of HBO.

At the tissue level, there are two theoretical advantages that may result from HBO: (i) each unit of blood is capable of delivering a greater absolute number of O_2 molecules, and (ii) there is an increase in tissue oxygen partial pressure particularly at the arterial end of the capillary (Fife & Camporesi, 1991). As movement of O_2 across the extravascular space is dependent on tissue oxygen gradients, a high P_aO_2 enlarges the cone of tissue around a single capillary that is protected from hypoxia.

Blood flow in most body tissues is adjusted in proportion to local oxygen demand - a phenomenon known as autoregulation (Fife & Camporesi, 1991). Oxygen has a direct effect in regulating vessel lumen size such that, on exposure to high partial pressures of oxygen (greater than 500 mmHg), pronounced vasoconstriction occurs in both arterial and venous vascular beds of most tissues of the body, with the exception of the lungs (Plewes & Farhi, 1983; Fife and Camporesi, 1991). This results in a marked reduction of blood flow to various organs of the body, such as the brain (Ohta *et al.*, 1990; Bergo & Tyssebotn, 1992), myocardium (Savitt *et al.*, 1994; Muhvich *et al.*, 1992), kidneys (Muhvich *et al.*, 1992; Hahnloser *et al.*, 1966), eyes (Anderson, 1967) and also splanchnic areas (Hammarlund, 1994). However, the increase in arterial oxygen content more than compensates for this reduction in total blood flow so that the net result is a transient increase in oxygen delivery and hence tissue oxygenation (Fife & Camporesi, 1991). It is the ability of HBO to fully oxygenate tissues in the presence of vasoconstriction that makes it clinically beneficial in conditions where edema is an underlying pathology (discussed further below).

There are a number of additional physiologic consequences of oxygen-induced vasoconstriction including an increase in peripheral vascular resistance, a decrease in heart rate and an increase in mean arterial pressure (Plewes & Farhi, 1983; Eggers *et al.*, 1962). The end result is a 10-20% reduction in cardiac output in man (Bassett & Bennett, 1977). It is likely that hyperbaric pressure and hyperoxia contribute independently to these phenomena (Eggers *et al.*, 1962; Weaver & Howe, 1994).

(2.3) Effects of HBO on the pulmonary system and ventilatory responses

Acute exposure to hyperoxia at pressures greater than 1 ATA has been shown to reduce afferent carotid chemoreceptor activity in animals (Fife & Camporesi, 1991; Torbati *et al.*, 1993). Also, under conditions where HBO results in venous blood haemoglobin being 100% saturated with oxygen (at treatment pressures of 2.8 ATA and above) there is a rise in venous blood pCO_2 resulting in a slight acidosis (Fife & Camporesi, 1991; Torbati *et al.*, 1993). The latter effect is due to the fact that, under normobaric conditions, a significant fraction of CO_2 is transported within the blood bound to deoxyhaemoglobin as the carbamino compound $HbCO_2$. If haemoglobin is fully saturated with O_2, this CO_2 must be transported in plasma. The result of these two opposing actions on respiratory drive (reduced afferent activity and increased P_vCO_2) is a net increase in resting ventilation during HBO (Fife & Camporesi, 1991).

The increased density of gas in the hyperbaric environment has been found to increase pulmonary work of breathing even at pressures as moderate as 2 ATA (Fife & Camporesi, 1991). This usually does not present a problem to the healthy subject under resting conditions but may result in significant reductions in ventilation in an exercising subject relative to normobaric conditions. Pulmonary mechanical responses such as lung compliance are generally unaffected by acute HBO but pulmonary vascular responses may be markedly altered. Amin *et al.* (1993) found that exposure of rats to 100% O_2 at 2.8 ATA for 6 hours resulted in an attenuated increase in pulmonary vascular resistance on subsequent exposure to a hypoxic stimulus.

Continuous exposure to oxygen at 3 ATA for 3.5 hours (approaching the limits of central nervous system [CNS] oxygen tolerance) has been found to result in small but significant decrements in certain indices of both expiratory and inspiratory function at 2-4 hours post-exposure, including forced expiratory volume in one second (FEV_1) and forced expiratory flow at 25-75% of vital capacity (FEF_{25-75}) (Clark *et al.*, 1991). The changes in FEV_1 and FEF_{25-75} were attributed to narrowing of peripheral airways as a result of pulmonary oxygen poisoning (Clark *et al.*, 1991). The authors acknowledged that the ability to tolerate continuous exposure to 100% O_2 at 3.0 ATA was limited by CNS rather than pulmonary manifestations of O_2 toxicity. Under "normal" clinical HBO treatment protocols, significant decrements in pulmonary function are unlikely to occur (Clark *et al.*, 1991). Prolonged exposures (several hours or days) to moderate pressures of O_2 (0.5-2 ATA) are required to elicit significant symptoms of pulmonary oxygen toxicity or lung injury (Clark *et al.*, 1991; Suzuki, 1994).

(2.4) Effects of HBO on the endocrine system

Information regarding the endocrine effects of HBO is poorly documented in current literature. In rats, a functional stimulation of the hypothalamic-pituitary axis by HBO has been reported (Golinov, 1986) as has a decrease in testosterone release (Rockert & Haglid, 1983). In humans HBO has been found to have no effect on thyroid hormones (Rakhmatullin & Halley, 1981) and plasma catecholamine levels (Tremellen *et al.*, 1993). More recent studies investigating the effect of acute and chronic HBO on blood

polyamines, adrenocorticotrophin (ACTH) and β-endorphin have determined that acute HBO causes a significant increase in both ACTH and β-endorphin and that chronic HBO (for 10 days) causes an increase in the blood concentrations of the polyamines spermine and spermidine (Casti *et al.*, 1993; Vezzani *et al.*, 1991).

The findings of Casti *et al.* (1993) and Vezzani *et al.* (1991) are interesting as the increases in the opiate hormone β-endorphin observed during and after acute HBO may explain the psychological effects commonly seen with HBO. Patients have reported feeling better or "energized" on cessation of HBO (Potera, 1995). Sceptics of HBO therapy's role in sports medicine have argued that this effect may explain the positive anecdotal reports from injured/fatigued athletes regarding the rehabilitative efficacy of HBO (Potera, 1995).

(2.5) Effects of HBO on the immune system

The information that is currently available regarding the effect of HBO on the immune system in normal and pathological conditions is limited (Bitterman *et al.*, 1994). Interpretation of the studies in the literature is complicated by the use of different oxygen profiles and durations of therapy and also investigation of various immune components (Brenner *et al.*, 1999). Lee *et al.* (1993) found that HBO exerted both stimulatory and inhibitory effects on subsets of the immune system in mice depending on the partial pressure of oxygen, the cell type and the lymphatic organ studied. However, in general,

animal studies have pointed to an immunosuppressive effect of chronic HBO (Gadd *et al.*, 1990; Hansbrough *et al.*, 1980; Inamoto *et al.*, 1991; Saito *et al.*, 1991) though there are animal studies that have found no immunosuppressive effects (Hussmann *et al.*, 1994). Changes in the immune system of animals observed after chronic HBO which have been indicative of immunosuppression include decreased lymphocyte proliferation to mitogen stimulation (Gadd *et al.*, 1990), a decrease in circulating leukocytes and spleen weight (Hansbrough *et al.*, 1980), a reduction of IL-1 and PGE_2 production from macrophages (Inamoto *et al.*, 1991) and suppression of the development of B and helper T cells (Saito *et al.*, 1991). Acute HBO in rats has been found to result in rapid T-cell activation with marked shifts of CD4 and CD8 subsets (Bitterman *et al.*, 1994), a reduction in prostaglandin (PGE_2 and $PGF_{1\alpha}$) synthesis in brain tissue (Mialon & Barthélémy, 1993) and an increase in TNF-α secretion from macrophages (Lahat *et al.*, 1995).

It has been well documented that HBO facilitates the generation of the oxidative burst of polymorphonuclear (PMN) leukocytes and thus increases bacterial killing (Park, 1994). However, this effect is only evident during conditions of HBO - pre-exposure to HBO does not influence subsequent PMN function (Gadd *et al.*, 1990). In contrast to the stimulatory effect of HBO on PMN oxidative killing, phagocytic activity and adherence of macrophages were unchanged during exposure to HBO (Mehm & Pimsler, 1986).

Human studies have been contradictory. Feldmeier *et al.* (1987) failed to demonstrate any effect of daily exposure to HBO at 2.4 ATA for 90 minutes over an

extended period on the immune response of healthy human volunteers. In contrast, Ginaldi *et al.* (1991) reported reduced serum levels of the soluble IL-2 receptor and IL-2 after HBO as well as elevated serum CD8 levels, which generally pointed to suppression of the immune response. Bitterman *et al.* (1993) tested the effect of an acute exposure to 100% oxygen at 2.8 ATA for 90 minutes on the peripheral blood mononuclear cell population in healthy subjects. They found that HBO caused a significant transient decrease in the CD4/CD8 ratio, a significant increase in the percentage of monocytes and human lymphocyte antigen-DR-bearing cells (HLA-DR), and no change in the levels of natural killer, CD3 and B-cells in the peripheral blood immediately after exposure to HBO. These changes were rapid and reversible and the authors acknowledged that they only provided a partial and limited picture of the distribution of mononuclear cells in the body.

To summarize, the effect of HBO on immune function is far from clear at present. The use of different HBO treatment pressures and durations and the study of various immune parameters has contributed to the confusion. Brenner *et al.* (1999), in a recent review of the area, concluded that the immune changes induced by HBO were unlikely to be of significance to healthy individuals. However, they suggested that careful monitoring of the immune function of patients with compromised immune systems may be important to the success of treatment.

(2.6) Oxygen toxicity and free radical-mediated damage

It was first realized in the late 18th century that oxygen had potentially toxic properties when Scheele noticed that enriched oxygen atmospheres inhibited the growth of plants (Bostek, 1989). In 1878, Paul Bert published *La Pression Barometrique* in which he described the universal nature of oxygen toxicity, the variation in sensitivity among species and the involvement of the central nervous system in mammals suffering from such toxicity. The first major evidence of the clinical importance of oxygen toxicity emerged in 1952 when reports from England and Australia linked the incidence of retrolental fibroplasias (RLF) in premature infants to the duration of oxygen therapy (Bostek, 1989). Since the late 1960's, oxygen toxicity has been found to play a role in many disease processes.

There is now general agreement that exposure to high partial pressures of oxygen results in an increased rate of production of oxygen-derived free radicals in the blood and CNS (Narkowicz *et al.*, 1993; Torbati *et al.*, 1992; Clark, 1994). Increased concentrations of these free radicals overwhelms the body's antioxidant and free radical scavenger systems and results in oxidant damage to cell membranes (lipid peroxidation) and constituents. Initiation of lipid peroxidation by reactive oxygen species such as the hydroxyl radical (OH·) and singlet oxygen may greatly increase oxidant damage by propagation of chain reactions with inactivation of critical enzymes, further cellular membrane damage and possible oxidation of proteins as well as lipids (Clark, 1994).

This damage is usually manifested as either central nervous system, pulmonary or ocular oxygen toxicity. CNS oxygen toxicity can occur in patients breathing oxygen at pressures of 2.0 ATA or greater whereas pulmonary oxygen toxicity can occur after prolonged exposure to oxygen pressures of only 0.5 ATA (Clark, 1994). Progressive myopia occurs in some patients who receive daily 90- to 120-minute exposures at 2.0 to 2.4 ATA for various chronic disease states. All these manifestations are completely reversible in the early stages with cessation of oxygen therapy (Clark, 1994).

Paradoxically, HBO has been found capable of decreasing injury in conditions where oxidative injury is involved. Zamboni and coworkers (1990, 1993) have determined that HBO *decreases* evidence of free radical-mediated damage during reperfusion by reducing venular endothelial neutrophil adherence. It was hypothesized that this prevented entry of neutrophils into reperfused tissue and thus the subsequent release of reactive oxygen species. The mechanism by which HBO decreases neutrophil adherence during reperfusion is not clear. However, several hypotheses have been forwarded. Kaelin *et al.* (1990) found that exposure to HBO resulted in significant increases in the activity of the free radical scavenger superoxide dismutase (SOD) in ischaemic skin flaps. If superoxide is playing a role in neutrophil endothelial adhesion, then increased SOD activity may decrease this adherence. Thom & Elbuken (1991) studied lipid peroxidation in vitro and demonstrated that, in the presence of monounsaturated fatty acids, hyperoxia altered the free radical pathway in favour of hydroperoxyl ($HO_2\cdot$) radical formation. The authors proposed that these quenching

radicals underwent a termination reaction with organic lipid radicals to form nonradical products, thus significantly reducing lipid peroxidation. This stabilizing effect may reduce endothelial activation resulting in reduced neutrophil adherence. Through his work with carbon monoxide poisoning in rats, Thom (1993a, 1993b) has proposed that HBO interferes with the function of B_2 integrins (CD18 epitopes) which are molecules on the surface of neutrophils required for adherence to vascular endothelium. It was suggested that this resulted in a lack of release of neutrophilic proteases normally responsible for conversion of xanthine dehydrogenase to xanthine oxidase, an important step for the production of oxygen derived free radicals.

Further evidence to suggest that HBO has an inhibitory effect on neutrophils has also been provided by Staples *et al.* (1995) at the University of British Columbia. These investigators found that intermittent post-exercise HBO resulted in significantly reduced myeloperoxidase levels in the muscles of rats during days subsequent to damaging muscular exercise. Myeloperoxidase is an enzyme found in the azurophilic granules of neutrophils that causes the production of hypochlorite, an effective antibacterial agent (Bostek, 1989).

(3) Clinical Indications for Hyperbaric Oxygen Therapy

Many of the past uses of HBO have had little or no scientific support which has led some to describe it as a "therapy in search of diseases" (Tibbles & Edelsberg, 1996). There are, however, a number of clinical indications for HBO that have a scientific basis

(Undersea and Hyperbaric Medical Society, 1992) and these are outlined in Table 2.1 below (Tibbles & Edelsberg, 1996):

Table 2.1: Diseases for which hyperbaric oxygen is currently used.

Diseases for which the weight of scientific evidence supports hyperbaric oxygen as effective therapy:

Primary therapy:

- Arterial gas embolism
- Decompression sickness
- Exceptional blood-loss anaemia
- Severe carbon monoxide poisoning

Adjunctive therapy:

- Clostridial myonecrosis
- Compromised skin grafts and flaps
- Osteoradionecrosis prevention

Diseases for which the weight of scientific evidence suggests hyperbaric oxygen may be helpful:

Primary therapy:

- Less severe carbon monoxide poisoning

Adjunctive therapy:

- Acute traumatic ischaemic injury
- Osteoradionecrosis
- Refractory osteomyelitis
- Selected problem wounds
- Radiation-induced soft-tissue injury

Diseases for which the weight of scientific evidence does not support the use of hyperbaric oxygen, but for which it may be helpful:

Adjunctive therapy:

- Necrotizing fasciitis
- Thermal burns

(4) The Role of Hyperbaric Oxygen in Sports Medicine.

There have been two major areas of interest regarding the role of HBO in sports medicine. The first has been the question of whether HBO has an ergogenic effect on subsequent physical performance. The second area of interest has focussed upon the role of HBO in the recovery from fatigue and/or soft tissue sports injuries.

(4.1) The potential ergogenic effects of HBO on subsequent physical performance.

In the early 1990's, anecdotal reports began to emerge in the media that some athletes were undergoing HBO prior to sports participation in the belief that their subsequent performance would be improved (Potera, 1995). These reports caused concern within the hyperbaric medicine community as there was no convincing scientific evidence to support the use of HBO for this purpose. In 1994, the Undersea and Hyperbaric Medical Society published a press release stating that the use of HBO for performance improvement was without scientific basis and that controlled human studies of the role of HBO in sports medicine were needed.

Interest in HBO as an ergogenic aid was originally sparked by the belief that treatment prior to physical performance might somehow "force" more oxygen into the body which could then be called upon during activity. Some scientific investigators (Cabric *et al.*, 1991) suggested that oxygen retention may occur after acute exposure to HBO which could result in an ergogenic effect on subsequent aerobic exercise

performance under normoxic, normobaric conditions. However, these investigators failed to offer a convincing explanation as to the precise mechanism(s) involved and no scientific work has yet demonstrated oxygen retention after HBO. In fact, calculations reveal that, even under resting conditions, the excess oxygen that is physically dissolved in arterial plasma during HBO at 2 ATA would be consumed within seconds after resumption of air breathing at 1 ATA (see Appendix A). Therefore, it is difficult to envisage HBO having a beneficial effect on subsequent athletic performance.

Five studies (Banister *et al.*, 1970; Hoffmann *et al.*, 1990; Cabric *et al.*, 1991; Webster *et al.*, 1998; McGavock *et al.*, 1999) have addressed the issue of whether acute HBO has an effect on performance during subsequent exercise in normoxic, normobaric conditions. One study (Bacon *et al.*, 1995) has investigated the ability of HBO to enhance recovery from anaerobic exercise.

Banister *et al.* (1970) found that 70 minutes of resting exposure to 100% O_2 at 2 ATA resulted in lower absolute ventilation, lower submaximal oxygen consumption and reduced blood lactate during intense submaximal exercise performed 40 minutes later (compared to the same exercise load without pre-oxygenation). One limitation of this study was the small sample size (n=2) which prevented the use of statistics to empirically evaluate any change. One subject was classed as an "active athlete" and the other as merely a "trained" subject and the responses of the two subjects to the imposed experimental conditions differed substantially.

Hoffmann *et al.* (1990) determined that administration of 100% O_2 at 1.5 ATA to trained senior cyclists had no effect on blood lactate and time to exhaustion during a

subsequent cycle ergometer test at 70% of maximal power output. Unfortunately the length of HBO treatment was unspecified in this latter study as was the time elapsed between HBO exposure and exercise.

Cabric *et al.* (1991) reported that exposure to 100% O_2 for 60 minutes at 2.8 ATA resulted in increased treadmill time to exhaustion and maximal oxygen consumption (VO_2max) for at least 3 hours post-HBO in healthy untrained female physical education students. No clear explanation of the results was provided but inspection of the data suggested that the subjects were inexperienced at maximal exercise testing. Therefore, the increased maximal work rate and VO_2max could have been due in part to a learning effect and not a result of the prior HBO exposure. It is difficult to rationalize how prior exposure to HBO might increase subsequent VO_2max. If tissue oxygenation were improved after HBO as a result of retention of oxygen (as was suggested by the investigators), this would most logically decrease the requirement of oxygen from the atmosphere during subsequent exercise. During maximal exertion, higher power outputs might possibly be achieved as a result of this improved tissue oxygenation but atmospheric oxygen consumption would not be expected to increase.

Webster *et al.* (1998) investigated the effect of a one hour exposure to HBO at 2 ATA prior to performance of a maximal exercise test in trained cyclists. The major dependent measures examined were VO_2max, ventilation threshold, lactate threshold and muscle oxygenation. No significant changes were seen in any of these variables after exposure to HBO relative to baseline measures and hence no ergogenic effect was apparent.

McGavock *et al.* (1999) examined the acute effects of HBO treatment (90 minutes at 2.5 ATA) on recovery following a prolonged run and also its effects on subsequent aerobic performance (running economy, VO_2max and run time to exhaustion) in a trained population. No significant effect of HBO was seen for any of these conditions, corroborating the findings of Webster *et al.* (1998).

Bacon *et al.* (1995) investigated the effect(s) of HBO on performance and blood lactate metabolism following anaerobic exercise. On two separate days, eight female field hockey players were required to perform a strenuous anaerobic exercise protocol followed by 45 minutes of either HBO (100% oxygen at 2.0 ATA) or sham conditions within a hyperbaric chamber. Immediately after this, subjects were then required to complete a repeated Wingate performance trial. No significant differences were found between conditions for post-hyperbaric mean power outputs or blood lactate values. Therefore, the investigators concluded that HBO was ineffective for enhancing recovery from anaerobic exercise.

In summary, although the earlier work on the role of HBO as an ergogenic aid produced discrepant findings, it appears from more recent research that the use of HBO for this purpose is unjustified.

(4.2) The role of HBO in the recovery from soft tissue injuries.

Persuasive anecdotal evidence exists that demonstrates the efficacy of hyperbaric healing. Residents living at high altitudes have noticed that wounds heal more slowly

than at sea level, while people living in high pressure undersea habitats have observed that wounds heal faster than under normobaric conditions (Hunt & Pai, 1972). Through rigorous scientific research, it has since emerged that oxygen is a "critical nutrient" of the wound and plays a controlling role in the reparative process (LaVan & Hunt, 1990).

With the occurrence of a soft tissue injury, such as a muscle contusion, there is disruption of cells and blood vessels at the injury site. By mechanisms that are not entirely understood, there is initiation of an inflammatory response at the site of injury (Tidball, 1995) which not only serves a phagocytic function but also conditions the local environment in a way that governs the destiny of the wounded tissue (LaVan & Hunt, 1990). The release of inflammatory mediators (cytokines) by various cells results in local vasodilation and movement of fluid from the bloodstream into the tissues. There is a marked increase in metabolism at the inflammatory site, due largely to the migration of rapidly metabolizing leukocytes, especially neutrophils and macrophages, across capillary walls into the tissues (Tidball, 1995; LaVan & Hunt, 1990). This increase in metabolism occurs when oxygen requirements are least easily provided as a result of physical damage and an increase in tissue water and diffusion distances (LaVan & Hunt, 1990). Therefore local hypoxia is a common occurrence at the site of inflammation together with increased lactate concentrations and decreased pH, despite the hyperaemia caused by inflammation (LaVan & Hunt, 1990; Hunt, 1977). Hypoxia of less than 30 mmHg of oxygen at the site of inflammation inhibits healing (LaVan & Hunt, 1990).

Increasing oxygen delivery to a site of inflammation has been found to result in a more rapid resolution of inflammation and rate of healing of the original injury (LaVan &

Hunt, 1990). Increased rates of collagen synthesis and matrix deposition are typically seen as oxygen is critical for the hydroxylation of proline and lysine residues in nascent collagen (LaVan & Hunt, 1990; Hunt & Pai, 1972). In fact, Mehm *et al.* (1988) found that in-vitro oxygen tensions of about 80 mmHg (ie. double that achieved breathing air at normal atmospheric pressure) achieve the greatest rate of collagen formation. High local partial pressures of oxygen also have been shown to promote angiogenesis (neovascularization) into hypoxic spaces (LaVan & Hunt, 1990). A close relationship has been found between oxygen tensions and tissue tensile strength, total collagen deposition, total protein and total DNA at an injury site (Hunt & Pai, 1972).

In addition to the above potentially beneficial effects of increasing oxygen tensions in a region of injury or inflammation, HBO therapy has a number of other potential benefits for recovery from soft tissue injuries. As mentioned previously, HBO causes systemic vasoconstriction but oxygen delivery is maintained or even improved due to the greater content of oxygen in arterial blood (Fife & Camporesi, 1991). It has been proposed that this hyperoxia-induced vasoconstriction reduces capillary pressure which may then decrease edema (Staples & Clement, 1996; James *et al.*, 1993; James, 1993). There is evidence that HBO results in reduction of edema after tourniquet-induced ischaemia in rats (Nylander *et al.*, 1985) and in the treatment of compartment syndrome (Skyhar *et al.*, 1986), crush injuries (Mathieu *et al.*, 1990), plastic surgery (Davis & Hunt, 1989), burns (Cianci *et al.*, 1988) and "edematous wounds" (Dooley *et al.*, 1996). However, Bird & Telfer (1965) have reported that blood flow to the limbs is reduced by only 12 to 19% and hence it could be argued that vasoconstriction is unlikely to be the

primary mechanism in skeletal muscle edema reduction.

As mentioned above, a further potentially beneficial effect of HBO in the recovery from soft tissue injuries is its ability to prevent excess neutrophils from entering an injury site as a result of interference with their normal mechanisms of adhesion to vascular endothelium (Zamboni *et al.*, 1993; Thom, 1993a; Thom, 1993b; Staples & Clement, 1996). This blunting of neutrophil adhesion may prevent the release of oxygen free radicals into the tissues. It has been proposed that reactive oxygen species released from neutrophils may be responsible for causing further damage to tissues, thus exacerbating the inflammatory response and impeding the healing process (Staples & Clement, 1996). Thom (1993a, 1993b) has also proposed that the reduction in neutrophil adhesion results in prevention of the release of neutrophilic proteases into tissues which cause the conversion of xanthine dehydrogenase to xanthine oxidase - an enzyme whose action generates superoxide anions as a by-product. Finally, hypoxic conditions are known to encourage the conversion of xanthine dehydrogenase to xanthine oxidase (Sjödin *et al.*, 1990) and hence the hyperoxic conditions of HBO may be capable of antagonizing this process (James, 1993).

Given the above potential benefits of HBO therapy for the reduction of swelling, edema and enhanced healing capacity, it is not surprising that professional sports organizations have recently begun to show an interest in this form of therapy for the rehabilitation of injured athletes (Potera, 1995; James *et al.*, 1993; James, 1993). However, a scarcity of research on humans exists in this domain at present. James *et al.* (1993), in a study of professional soccer players in Scotland, reported that physiotherapy

combined with HBO resulted in a 70% reduction in days lost to injury compared to physiotherapy alone. The results were a comparison of a physiotherapist's estimation of the time-course for the injury and the actual number of days missed as a result of routine therapy and daily (1 hour) sessions of HBO at 2 ATA. This study lacked a control group and also input from an objective third party (such as a physician) for assessment of injury status. In addition, as this was a preliminary study, the injuries treated were extremely heterogeneous including tendonitis, sprains and muscle strains.

Staples (1999) utilized a randomized, double-blind design with a quantifiable injury to investigate the ability of intermittent HBO to enhance recovery from exercise-induced muscle injury/soreness in human subjects. Subjects performed intense isokinetic eccentric contractions of the quadriceps muscles of one leg (300 repetitions in 30 minutes). They were then treated daily over a 5 day period, receiving either a sham HBO treatment (normal oxygen [21%] at atmospheric pressure) or the HBO treatment (100% oxygen at 2.0 ATA). It was found that HBO enhanced the recovery of eccentric strength but had no effect on muscle soreness/pain as measured by a visual analogue scale. The author suggested that higher HBO treatment pressures should be used in future studies together with biochemical markers of muscle damage.

Harrison *et al.* (1999) also examined the role of HBO in the treatment of exercise-induced muscle injury. Subjects were split into three groups: a control group, immediate HBO (started 2 hours post-exercise) and delayed HBO (started 24 hours post-exercise). All groups performed the same exercise protocol on the forearm flexors designed to elicit muscle damage in that muscle group. HBO treatments consisted of daily exposures of

100 minutes at 2.5 ATA until day 4 post-exercise. Dependent variables included cross sectional area of the muscle group, T_2 relaxation via MRI (the transverse relaxation time of protons within the water in muscle), isometric strength, perceived soreness and serum creatine kinase. These were assessed at baseline, 24 hours, 7 and 15 days post-exercise. All dependent measures demonstrated significant changes after injury in every group but there were no differences between groups. This suggested that HBO was not effective in speeding recovery from exercise-induced muscle damage.

Borromeo *et al.* (1997) investigated the efficacy of HBO in the reduction of morbidity from acute ankle sprains. Patients that reported to the Temple University Sports Medicine Clinic less than 72 hours post injury were eligible to volunteer for the study. Thirty two subjects (20 males and 12 female) with acute ankle sprains were randomly assigned in a double blind manner to HBO or sham treatment groups. Subjects were administered three treatments within a seven day period of either HBO (100% oxygen at 2 ATA) or sham HBO (air at 1.1 ATA). Dependent measures were perceived ankle pain, edema, active and passive ranges of motion, ankle function and recovery time. Data were collected before and after all three HBO or sham treatments. It was found that HBO had no additional effect over the sham treatment on any of the dependent variables measured. Potential limitations of this study were the lack of a specific injury definition and the fact that the subjects were allowed to pursue a standard home rehabilitation program in addition to the HBO/sham treatments. Also, it was not possible to standardize the precise timing of the HBO/sham treatments with respect to the time of injury. The patients averaged 34 hours from the time of injury to their appearance in the sports

medicine centre and in some cases the first HBO session occurred several days after the time of injury. Staples & Clement (1996) have suggested that HBO should be administered within hours after the initial injury for HBO to be maximally effective. Also, the authors suggested the use of higher pressures during HBO treatment in further studies.

Best *et al.* (1998) examined the use of HBO therapy after an acute muscle stretch injury to the tibialis anterior in rabbits. Treatments began 24 hours after injury and consisted of 60 minutes at 2.5 ATA daily for 5 days. Evaluation of the injury 7 days post indicated that the animals subjected to HBO possessed a significantly smaller strength deficit between the injured and non-injured limbs and also morphologic studies revealed more complete healing in the treated group. Therefore, the results suggested that HBO may play a role in accelerating recovery after acute muscle stretch injury.

Two abstracts have been published in the literature investigating the rehabilitative effects of HBO on soft tissue "sports-like" injuries in animals. As outlined above, Staples *et al.* (1995) investigated the effects of HBO treatment on the levels of biochemical muscle metabolites in rats after downhill running. They demonstrated that the muscles of rats treated with HBO (1 hour at 2 ATA per day for 5 days) showed significantly reduced levels of myeloperoxidase - a result suggestive of an inhibitory effect of HBO on the inflammatory process or the ability of HBO to actually modulate the injury to a tissue. Webster *et al.* (1996) demonstrated that exposure of rats to HBO (5 daily exposures for 90 minutes at 2.8 ATA for 8 weeks) resulted in enhanced recovery of ligament strength and stiffness relative to a group of untreated rats.

It has been stated that it is important to administer HBO as soon as possible (within the first 4-6 hours) after injury (Jain, 1990; Staples & Clement, 1996). It is also apparent that the benefits of HBO on injury healing will only be realised if the injury site has an intact vascular supply (LaVan & Hunt, 1990). Even hyperbaric hyperoxic conditions cannot force oxygen into severely ischaemic tissue, underlining the important role of adequate perfusion to an injury (LaVan & Hunt, 1990).

To summarize, research has demonstrated that hyperbaric oxygen therapy has various physiological effects that could feasibly have an influence on the recovery from soft tissue "sports" injuries. However, there has been little rigorous scientific testing in a sports medicine setting and the few studies that have been performed have been discrepant in their findings. At present, claims about the effectiveness of hyperbaric oxygen therapy in the field of sports medicine are almost entirely anecdotal.

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CHAPTER THREE

STUDY 1: EXERCISE AFTER ACUTE HYPERBARIC OXYGENATION: IS THERE AN ERGOGENIC EFFECT?

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Abstract

The purpose of this study was to determine the effects of a one hour exposure to 2.0 atmospheres pressure (2 ATA, 202.6 kPa) and 100% oxygen (O_2) on subsequent maximal oxygen consumption ($\dot{V}O_2max$), ventilation threshold (VT), lactate threshold (LT) and muscle oxygenation (%Mox) during incremental exercise to maximum on a cycle ergometer. Two baseline exercise tests (T1 and T2) were performed on separate occasions without prior exposure to hyperbaric oxygen (HBO) and a third test (T3-HBO) was performed after (22.5 ± 5.6 minutes) HBO. Near infra-red spectroscopy (NIRS) was used to monitor oxygenation of the left vastus lateralis muscle during T2 and T3-HBO. No significant differences were observed between VO_2max, VT or LT among any of the exercise tests. There was no significant difference in %Mox between T2 and T3-HBO except at 235 Watts where there was a significant elevation in %Mox during T3-HBO relative to T2. These results suggest that prior exposure to HBO (100% O_2 at 2 ATA for one hour) has no ergogenic effect on subsequent incremental exercise performance.

***Key words**: maximal oxygen consumption, anaerobic threshold; near infra-red spectroscopy; muscle oxygenation; performance; hyperoxia.*

Introduction

In recent years there has been much interest in hyperbaric oxygen (HBO) in the field of sports medicine (James, 1993; Potera, 1995). Several North American professional sports teams have purchased hyperbaric chambers in the belief that HBO treatment will help athletes recover more quickly from injury and strenuous exercise (James, 1993; Potera, 1995). There have also been reports of professional athletes breathing HBO prior to participation in their respective sports in the belief that subsequent performance will be improved (Potera, 1995). However, there is little convincing scientific evidence to support the efficacy of HBO for these purposes. In 1994, the Undersea and Hyperbaric Medical Society published a press release stating that the use of HBO for performance improvement was without scientific basis and that controlled human studies of the role of HBO in sports medicine were needed.

Some investigators (Cabric *et al.*, 1991) have suggested that oxygen may be retained by the body after acute exposure to HBO and that there may be an ergogenic effect on subsequent aerobic exercise performance under normoxic, normobaric conditions. However, these investigators failed to offer a convincing explanation as to the precise mechanism(s) involved and the present authors could find no scientific work demonstrating oxygen retention after HBO. Calculations reveal that, even under resting conditions, the excess oxygen that is physically dissolved in arterial plasma during HBO at 2 ATA would be consumed within seconds after resumption of air breathing at 1 ATA (see Appendix A). Therefore, it is difficult to envisage HBO having a beneficial effect on

subsequent athletic performance.

Only three studies (Cabric *et al.*, 1991; Banister *et al.*, 1970; Hoffmann *et al.*, 1990) have addressed the issue of whether acute HBO has an effect on performance during subsequent exercise in normoxic, normobaric conditions. Results of these studies have been discrepant with two (Cabric *et al.*, 1991; Banister *et al.*, 1970) suggesting enhanced performance and one (Hoffmann *et al.*, 1990) finding no effect. All studies performed thus far have suffered from a number of methodological limitations. Clearly the effect of acute HBO on subsequent normobaric, normoxic performance deserves clarification, especially in the light of current interest in its use in the field of sports medicine and reports of its use for ergogenic purposes.

Therefore, the purpose of the present study was to determine the acute effects of a one hour exposure to HBO (100% O_2 at 2 ATA, 202.6 kPa) on maximal oxygen consumption (VO_2max), ventilation threshold (VT), lactate threshold (LT) and muscle oxygenation (%Mox) during incremental exercise on a cycle ergometer in trained cyclists. In light of the limitations of the work done in this area to date, it was hypothesized in the present study that there would be no effect of acute HBO on the performance variables outlined above.

Methods

Subjects

Twelve trained endurance cyclists, experienced in maximal exercise testing, were recruited from the University of Alberta and local Edmonton cycling clubs. All subjects signed informed consent forms (see Appendix B) and the research was approved by the Faculty of Physical Education and Recreation Research Ethics Committee at the University of Alberta and also the Research Steering Committee of the Caritas Health Group, Edmonton. All subjects underwent a medical examination by a physician prior to entry into the hyperbaric chamber to verify that it was safe for them to undergo hyperbaric oxygenation. Contra-indications to HBO have been discussed previously (Jain, 1990). Table 3.1 contains the subjects' descriptive data.

Subjects were allowed to maintain their training regimes during the period of the study but were requested to refrain from heavy exercise for the 24 hour period prior to data collection. Subjects were also requested not to change dietary habits and to maintain adequate hydration during the period of the study and to avoid drinking caffeinated or alcoholic beverages during the 24 hours preceding data collection.

Experimental design

The present study was a quasi-experimental, time series design. Each subject

performed three exercise tests (T1, T2, T3-HBO). These tests were performed on separate days and at approximately the same time of day to minimise variability due to circadian rhythms. The first two exercise tests (T1, T2) were designed to establish each subjects' baseline data. The third test (T3-HBO) was preceded by the treatment (HBO). There was no separate control or placebo group due to financial and time constraints on the use of the hyperbaric chambers.

Exercise test procedures

Incremental exercise tests to assess VO_2max, VT and LT were performed on a Monark cycle ergometer (Model 818) on three separate occasions (T1, T2, T3-HBO). T1 and T2 were baseline tests performed 10 and 4 days respectively prior to exposure to hyperbaric oxygen (HBO) and T3-HBO was performed as soon as possible (22.5 ± 5.6 minutes) after cessation of HBO. The 22.5 ± 5.6 minute period was due to the fact that subjects needed to change clothing after HBO to prepare for the exercise test and also they had to be fitted with the near infra-red spectrometer and undergo a short rest period prior to exercise to ascertain resting muscle oxygenation (outlined below).

Expired gases were assessed during the exercise tests using a Horizon metabolic measurement cart that provided data averaged over 15 second intervals (SensorMedics, California) and heart rate was monitored every minute using a Polar heart rate monitor (Polar Electro, Finland). The test protocol started at a power output of 157 Watts (2.0kp @ 80 rpm) and was increased by approximately 40 Watts (0.5kp) every 3 minutes until

VT (defined as a breakaway in the V_E/VCO_2 ratio from a minimum value) was surpassed. Power output was then increased by 40 Watts (0.5kp) every minute until volitional exhaustion occurred. The timing of the power output increments was established during T1 for each subject and was purposefully kept identical for that subject during T2 and T3-HBO. Identical equipment was used to test the subjects for all three exercise tests.

Near infra-red spectroscopy

Near infra-red spectroscopy is based on the principle of differential absorption properties of oxygenated and deoxygenated forms of haemoglobin and myoglobin in the near infra-red range (ie. at wavelengths between 760nm and 850nm of the absorption spectrum) (Balardinelli *et al.*, 1995; Mancini *et al.*, 1994). Monitoring of the tissue absorbance at these two wavelengths allows trends in haemoglobin oxygenation in the small blood vessels (arterioles, capillaries, venules) and possibly oxygenation of tissue myoglobin to be observed noninvasively (Mancini *et al.*, 1994). As the absolute path length of the NIR light cannot be ascertained it is not possible to measure absolute levels of haemoglobin and myoglobin oxygenation. Oxygenation levels must be scaled to the overall change in signal obtained for each individual to arrive at a percentage value (outlined below).

A commercially available near infra-red (NIR) spectrometer (Runman, NIM Inc., PA.) was positioned over the left vastus lateralis muscle 160-190mm from the superior aspect of the patella, parallel to the major axis of the thigh, and was used to monitor

tissue oxygenation of this muscle during T2 and T3-HBO. The position of the probe for each individual was noted after T2 so that it would be replicated exactly for T3-HBO. A piece of clear plastic wrap was placed between the probe's photodetectors and the skin to prevent distortion of the signal due to the accumulation of sweat during exercise. The NIR unit was interfaced with an A/D board to record and process the infra-red signal during the exercise tests. The signal was recorded at a sampling frequency of 30 Hz by the A/D board and a customized software program was used to display the NIR signal on a computer monitor during the tests.

Prior to each test the NIR instrument was calibrated at 760nm and 850nm as recommended by the manufacturer. The NIR signal was recorded continuously during the test along with respiratory gas exchange data. Upon completion of the test, the raw NIR signal was averaged over 15 sec intervals for the entire duration of the test, including the rest period before and the recovery period after each exercise test. Muscle oxygenation, expressed as a percentage of the maximum physiological range possible (%Mox), was calculated using the following formula (Balardinelli *et al.*, 1995):

$$\%Mox = (y - M) \times 100 / (m - M)$$

where y is the absorbency observed at rest or during exercise, M is the minimum absorbency recorded (at maximal exercise), and m is the maximal absorbency attained (after cessation of exercise). Mean %Mox was calculated: (a) during a two minute rest period immediately prior to exercise with the subject relaxed and stationary while seated

on the cycle ergometer, (b) during each workload of the exercise test and (c) for a five minute recovery period immediately after cessation of exercise (the subject was allowed to perform active recovery at 0W for three minutes and then was requested to stop and sit quietly on the cycle ergometer for a further two minutes).

Blood sampling, preparation and analysis

A 20 gauge teflon catheter was placed in an antecubital vein prior to each exercise test and 1-2 ml of blood was drawn at rest, during the last 30 seconds of each 3 minute workload and also 5 minutes after completion of the test. The catheter was kept patent between samples by flushing with 0.5 ml heparin lock flush solution (100 USP Units/ml). To ensure that subsequent blood samples were not contaminated with heparin, 1-2 ml of blood was drawn and discarded before every blood sample was collected. Samples of 0.5 ml whole blood were immediately deproteinized in 2.0 ml of chilled 4% perchloric acid, centrifuged at 3000 xg for 10 minutes and the supernatant was stored at -70°C. Venous blood lactate concentration ($mmol \cdot L^{-1}$) was determined using the spectrophotometric method of Sigma (#826-UV, Sigma Chemical Company, St. Louis). A small amount of resting whole blood was taken in a capillary tube and centrifuged for subsequent determination of haematocrit using a micro-capillary reader (International Equipment Company, Needham Hts., MA).

Determination of VO_2max and lactate and ventilatory thresholds

The main criteria for VO_2max was the attainment of a peak and plateau in VO_2 during the last exhaustive portion of the exercise test (Thoden, 1991). A plateau was deemed to occur when oxygen uptake levelled off (an increase of less than 0.100 $L \cdot min^{-1}$) despite increasing power output. Secondary criteria included a RER in excess of 1.10, attainment of known or age-predicted maximal heart rate and volitional exhaustion (Thoden, 1991).

VT for each test was determined by graphing the average V_E/VCO_2 ratio of each workload against power output (Bhambhani & Singh, 1985). Three experienced observers independently indicated the power output at which the lowest average V_E/VCO_2 ratio occurred prior to a systematic increase in V_E/VCO_2 with increasing power output. VO_2 was then averaged over the last minute of this power output to determine VO_2 at VT in $L \cdot min^{-1}$. If one observer disagreed on the power output of lowest V_E/VCO_2 then the estimate of the two other investigators was taken (there was no occurrence of all three observers disagreeing). LT was identified by determining the power output immediately prior to that which elicited an increase in blood lactate concentration of greater than $1 mmol \cdot l^{-1}$ (Thoden, 1991). VO_2 was averaged over the last minute of this power output to determine VO_2 at LT in $L \cdot min^{-1}$.

Hyperbaric oxygenation protocol

HBO exposure was performed in a Sechrist monoplace hyperbaric chamber (Sechrist Industries Inc., Anaheim, CA.) under the supervision of trained medical personnel at the Misericordia Hospital, Edmonton. Subjects were compressed to 2 ATA (202.6 kPa) over a period of 15 minutes whereupon the chamber was filled with 100% O_2 for a duration of 60 minutes. At the end of the 60 minute period 15 minutes were allowed for decompression to atmospheric pressure. The protocol was employed as (a) it elicited a partial pressure of O_2 that posed little risk to the subjects and (b) it is very similar to that typically used by athletes who have access to hyperbaric chambers (Staples & Clement, 1996).

Statistical analysis

Means and standard deviations are reported and repeated measures one-way (time) ANOVA on each dependent variable was used to determine any differences between test scores. Multiple comparison procedures for significant F ratios were performed using a Scheffé analysis. Alpha was set *a priori* at $p<0.05$.

Results

The exercise test results are shown in Table 3.2. No significant differences were

observed for VO_2max, VT, LT, maximal ventilation and maximal heart rates among any of the exercise tests.

Mean blood lactate concentrations during individual workloads of the tests are shown in Table 3.3. Each value in the table represents the mean of all twelve subjects except the values at 314 W where n=7. Five of the twelve subjects were deemed to have passed VT during workload 4 (275 W) and hence the last blood sample was drawn at the end of this workload. The remaining seven subjects surpassed VT at 314 W where the last blood sample was drawn. Significant differences were found in mean blood lactate at 157W, 196W and 235W between T1 and T2.

Table 3.4 shows the mean VO_2 and VCO_2 data for every workload of T1, T2, and T3-HBO including three minute and one minute workloads. Three subjects completed a total of six workloads (ie. power output at VO_2max corresponded to 353 W), three subjects completed seven workloads (power output at VO_2max corresponded to 392 W) and six subjects completed a total of eight workloads (power output at VO_2max corresponded to 432 W). A significant difference in mean VO_2 was found between T1 and T2 at 196 W. Also, VO_2 during T2 was significantly different from T1 and T3-HBO at 235 W, 275 W and 314 W. A significant difference in mean VCO_2 was found between T1 and T2 at 275W. VCO_2 during T1 was also significantly different from T2 and T3-HBO at 196W, 235W, 314W and 353W.

No significant differences were found between workloads of the baseline tests (T1 and T2) and T3-HBO for mean ventilation, heart rates and respiratory exchange ratios (RER).

Resting haematocrits immediately prior to each exercise test were 47.8 ± 1.8, 44.6 ± 1.2 and 47.0 ± 3.2 percent respectively for T1, T2 and T3-HBO. Haematocrits prior to T2 were significantly lower than those prior to T1 and T3-HBO.

Figure 3.1 charts the mean percent muscle oxygenation for T2 and T3-HBO at rest, during six workloads of the exercise tests (every subject completed at least six workloads) and during recovery. A significant difference between T2 and T3-HBO was found only at 235 W with %Mox during this workload being significantly greater during T3-HBO.

Discussion

The primary purpose of this study was to investigate the effects of a one hour exposure to HBO (100% O_2 at 2 ATA) on VO_2max, VT, LT and %Mox during subsequent incremental cycle ergometer exercise performed under normal atmospheric conditions. There were no significant differences after HBO for VO_2max, VT or LT suggesting that prior exposure to HBO at the pressure and duration used had no ergogenic effect on subsequent incremental exercise performance. Also, there were no significant differences in %Mox with and without prior exposure to HBO except at one power output (235 W) suggesting that HBO was incapable of consistently elevating %Mox during subsequent incremental exercise. Thus our hypothesis that prior acute HBO would have no ergogenic effect on incremental exercise performance was supported.

The absence of an increase in VO_2max after prior HBO in the present study

contrasted with the results obtained by Cabric *et al.* (1991). These investigators reported that exposure to 100% O_2 for 60 minutes at 2.8 ATA resulted in increased treadmill time to exhaustion and VO_2max for at least 3 hours post-HBO in healthy untrained female physical education students. No clear explanation of the results was provided but inspection of the data suggested that the subjects may possibly have been inexperienced at maximal exercise testing. Therefore, the increased maximal work rate and VO_2max could have been partly a learning effect and not a result of the prior HBO exposure. It is difficult to rationalize how prior exposure to HBO might increase subsequent VO_2max. If tissue oxygenation were improved after HBO as a result of retention of oxygen [as was suggested by Cabric *et al.* (1991)], this would most logically decrease the requirement of oxygen from the atmosphere during subsequent exercise. During maximal exertion, higher power outputs might possibly be achieved as a result of this improved tissue oxygenation but atmospheric oxygen consumption would not be expected to increase, especially at similar power outputs.

The results of the present study are also in disagreement to those of Banister *et al.* (1970). These investigators found that 70 minutes of resting exposure to 100% O_2 at 2 ATA resulted in lower absolute ventilation, lower VO_2 and reduced metabolic acidosis during intense submaximal exercise performed 40 minutes later (compared to the same exercise load without pre-oxygenation). One limitation of this study was the small sample size (n=2) which prevented the use of statistics to empirically evaluate any change. One subject was classed as an "active athlete" and the other as merely a "trained" subject and the responses of the two subjects to the imposed experimental

conditions differed substantially.

In contrast to the results of Banister *et al.* (1970), mean submaximal VO_2 in the present study was not reduced after acute HBO relative to the exercise tests without prior HBO (table 3.4). In fact, mean VO_2 during several workloads of the second exercise test (T2) was significantly lower than either of the other tests (T1 and T3-HBO). These differences are difficult to explain as conditions during both baseline tests were identical for each subject. It was ensured that subjects were adequately rested prior to all tests and systematic instrument error was not evident upon careful scrutiny. The differences could not be attributed to a difference in substrate utilization as the RER values during each of the tests showed no significant differences.

The mean submaximal VCO_2 data (table 3.4) demonstrated a similar pattern between tests to the VO_2 data. In general, the lowest values were obtained during T2, intermediate values were obtained during T3-HBO and the highest values were obtained during T1, where several were significantly higher than T2 and T3-HBO. The mechanism(s) underlying these differences were unclear. It should be noted that the values of submaximal VCO_2 and VO_2 during T3-HBO fell between those of the two baseline tests which would imply that HBO had little effect on submaximal exercise performance.

Again in contrast to the results of Banister *et al.* (1970), blood lactate concentration was not reduced in the present study after HBO relative to the baseline tests suggesting that there was no difference in the degree of metabolic acidosis during exercise after HBO. There were significantly lower blood lactates between T1 and T2

during several submaximal workloads (table 3.3) that could have been related to the lower haematocrit found prior to T2 (ie. possible haemodilution, explained below). The precise cause of this lower haematocrit was unclear though it could have been produced by two possible mechanisms. There could have been an increase in plasma volume (hypervolemia) due to an increase in hydration of the subjects (or some other unknown factor) or there could have been a decrease in erythrocyte volume in the bloodstream, the latter mechanism being the least plausible. Of course, it is possible that the two mechanisms may have occurred simultaneously. If hypervolemia was a contributor to the reduced haematocrit this might explain, at least in part, the lower blood lactate values observed during T2. In effect, a larger blood volume could have "diluted" the lactate that had effluxed from working muscle.

The lack of a difference in blood lactate after HBO in the present study also contrasted with the results of Weglicki *et al.* (1966). These investigators found that exercise performed by dogs 45 minutes after exposure to 100% O_2 at 3 ATA resulted in significantly lower production of blood lactate relative to exercise without prior hyperoxia. Haematocrits were not reported in this study so haemodilution cannot be ruled out as a cause of the lower lactates. The authors believed the most likely explanation for this result was the inhibition and inactivation of glycolytic sulphydryl enzymes that persisted after HBO. The results of the present study do not support this finding though it is possible that the use of 2 ATA pressure was not sufficient to elicit an inhibitory effect on glycolytic enzymes. It should be noted that, in exercise physiology, lower blood lactate is often interpreted as a positive adaptation to exercise stress.

However, lower blood lactate due to glycolytic enzyme inhibition is more likely to be ergolytic rather than ergogenic as the substrate flux rate through glycolysis would be slowed.

A commonly observed cardiovascular effect of hyperbaric hyperoxia has been transient bradycardia, during rest and exercise (Fife & Camporesi, 1991). However, there were no differences in heart rate during individual test workloads or maximal heart rate in the present study suggesting that any bradycardic effect of hyperbaric oxygen did not persist during exercise after HBO.

The results of the present study are in agreement with those of Hoffmann *et al.* (1990). These investigators determined that administration of 100% O_2 at 1.5 ATA to trained senior cyclists had no effect on blood lactate and time to exhaustion during a subsequent cycle ergometer test at 70% of maximal power output. Unfortunately the length of HBO treatment was unspecified in this latter study as was the time elapsed between HBO exposure and exercise.

Mean relative muscle oxygenation (%Mox) during T2 and T3-HBO was not statistically different except at 235W (figure 1). At this power output %Mox was greater during T3-HBO relative to T2. Muscle oxygenation during rest and recovery were not significantly different for the two tests. It is difficult to explain the mechanisms underlying the difference in %Mox at 235W but it was possible that HBO caused a supersaturation of myoglobin in muscle. It is also known that hyperbaric hyperoxia causes general vasoconstriction (Fife & Camporesi, 1991). It is feasible that following HBO there was a reactive vasodilatation which may have caused the higher %Mox

(Cabric *et al.*, 1991). However, if these above possibilities occurred then %Mox should have been significantly elevated during rest and other workloads also. There was no difference in the mean ventilation at 235W during both tests and so the observed difference cannot be attributed to hyperventilation-induced lowering of arterial CO_2 levels (P_aCO_2). Lower P_aCO_2 would have encouraged a leftward shift in the oxygen dissociation curve and an increase in %Mox as haemoglobin would have a higher affinity for oxygen. It is possible that the lower %Mox observed at 235W during the test without prior HBO (T2) may have been related to the significantly lower haematocrit observed prior to this test (44.6% for T2 versus 47.0% for T3-HBO). If this lower haematocrit was partially due to a reduction in erythrocyte volume this may have produced a relative deficit in oxygen delivery to working muscle which could have contributed to the lower %Mox observed during T2. This potential mechanism is highly speculative, however, and does not fully explain why a difference was detected at one power output only.

It is quite possible that the time period in the present study between HBO and exercise (22.5 ± 5.6 minutes) was too long to observe an ergogenic effect. However, this would disagree with the study of Cabric *et al.* (1991) who determined that an ergogenic effect persisted for at least three hours after HBO. It is suggested that future studies shorten the time that elapses between HBO exposure and exercise in case there is a potential advantage until a certain time post HBO. It is also possible that the pressure and duration of treatment used in the present study were not sufficient to elicit an ergogenic effect. Further research should experiment with higher pressures and longer durations of HBO before potential ergogenesis can be ruled out. However, theoretical calculations

suggest that no ergogenic effect should occur regardless of the treatment protocol employed or the time between HBO exposure and subsequent performance.

Conclusions

To conclude, an acute one hour exposure to 100% O_2 at 2 ATA had no ergogenic effect on subsequent incremental exercise performed under normoxic, normobaric conditions. The present findings lend support to the statement of the Undersea and Hyperbaric Medical Society that the use of HBO for ergogenic purposes is without scientific basis.

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Table 3.1. Subject Characteristics (n=12).

Age (yr)	Height (cm)	Body Mass (kg)	VO_2max ($L \cdot min^{-1}$)	VO_2max ($ml \cdot kg^{-1} \cdot min^{-1}$)
25.0 ± 5.1 (20 - 40)	178.5 ± 4.0 (174 - 189)	73.8 ± 4.7 (64.3 - 79.5)	4.6 ± 0.3 (4.1 - 4.9)	62.3 ± 3.8 (54.4 - 70.5)

Data are means ± SD, ranges are reported in parentheses.
VO_2max = maximal oxygen consumption.

Table 3.2. Exercise test results.

Test	VO_2max ($L \cdot min^{-1}$)	VT ($L \cdot min^{-1}$)	LT ($L \cdot min^{-1}$)	$V_E$max ($L \cdot min^{-1}$)	HRmax (bpm)
T1	4.64 ± 0.25	3.60 ± 0.38	3.28 ± 0.45	197.2 ± 28.9	195.2 ± 8.6
T2	4.47 ± 0.24	3.46 ± 0.30	3.27 ± 0.37	190.3 ± 23.4	194.4 ± 8.0
T3-HBO	4.63 ± 0.32	3.47 ± 0.31	3.21 ± 0.25	198.7 ± 28.5	195.2 ± 8.5

Data are means ± SD.
VO_2max = maximal oxygen consumption; VT = ventilatory threshold (expressed as a value of VO_2); LT = lactate threshold (expressed as a value of VO_2); $V_E$max = maximal ventilation; HRmax = maximal heart rate.

Table 3.3. Blood lactate concentrations during rest, individual test workloads and 5 minutes post-exercise.

Test workload (W)	T1	T2	T3-HBO
Rest	0.91 ± 0.46	0.85 ± 0.44	0.74 ± 0.28
157	1.08 ± 0.44	0.77 ± 0.33[a]	0.93 ± 0.29
196	1.75 ± 0.68	1.35 ± 0.45[a]	1.47 ± 0.51
235	3.08 ± 1.36	2.40 ± 1.08[a]	2.58 ± 1.24
275	4.73 ± 2.01	4.36 ± 1.91	4.39 ± 1.83
§314	5.43 ± 1.06	4.86 ± 1.11	5.34 ± 1.27
Recovery	10.51 ± 3.01	10.49 ± 2.51	10.84 ± 3.25

Data are means ± SD ($mmol \cdot l^{-1}$).
a - significant difference between T1 and T2 ($p<0.05$).
§ N=12 except at 314 W where n=7.

Table 3.4. VO_2 and VCO_2 during individual test workloads.

Test workload (W)*	Variable	T1	T2	T3-HBO
157	VO_2	2.33 ± 0.10	2.23 ± 0.09	2.31 ± 0.12
	VCO_2	2.15 ± 0.16	2.02 ± 0.14	2.08 ± 0.14
196	VO_2	2.86 ± 0.11	2.71 ± 0.10[a]	2.81 ± 0.13
	VCO_2	2.77 ± 0.16[a,c]	2.61 ± 0.14	2.66 ± 0.13
235	VO_2	3.33 ± 0.10	3.18 ± 0.08[a, b]	3.29 ± 0.09
	VCO_2	3.37 ± 0.22[a,c]	3.18 ± 0.13	3.24 ± 0.19
275	VO_2	3.79 ± 0.12	3.65 ± 0.11[a, b]	3.77 ± 0.10
	VCO_2	4.02 ± 0.30[a]	3.83 ± 0.21	3.90 ± 0.26
314	VO_2	4.18 ± 0.16	4.03 ± 0.18[a, b]	4.17 ± 0.13
	VCO_2	4.69 ± 0.30[a,c]	4.46 ± 0.24	4.51 ± 0.23
353	VO_2	4.37 ± 0.28	4.23 ± 0.26	4.37 ± 0.17
	VCO_2	5.10 ± 0.21[a,c]	4.83 ± 0.23	4.87 ± 0.27
§392	VO_2	4.57 ± 0.24	4.40 ± 0.28	4.56 ± 0.25
	VCO_2	5.44 ± 0.38	5.20 ± 0.38	5.34 ± 0.40
§432	VO_2	4.57 ± 0.23	4.41 ± 0.22	4.69 ± 0.33
	VCO_2	5.38 ± 0.61	5.33 ± 0.16	5.52 ± 0.38

Data are means ± SD ($L \cdot min^{-1}$, STPD).

a - significant difference between T1 and T2 ($p<0.05$).

b - significant difference between T2 and T3-HBO ($p<0.05$).

c - significant difference between T1 and T3-HBO ($p<0.05$).

§ N=12 except at 392 W (n=9) and 432 (n=6).

* Values obtained for the power outputs from 157W to 275W represent data averaged over 3 minutes (n=12), values for the power outputs from 353W to 432W represent data averaged over 1 minute (n=12) and the values at 314W represent data averaged over 3 minutes (n=7) and 1 minute (n=5).

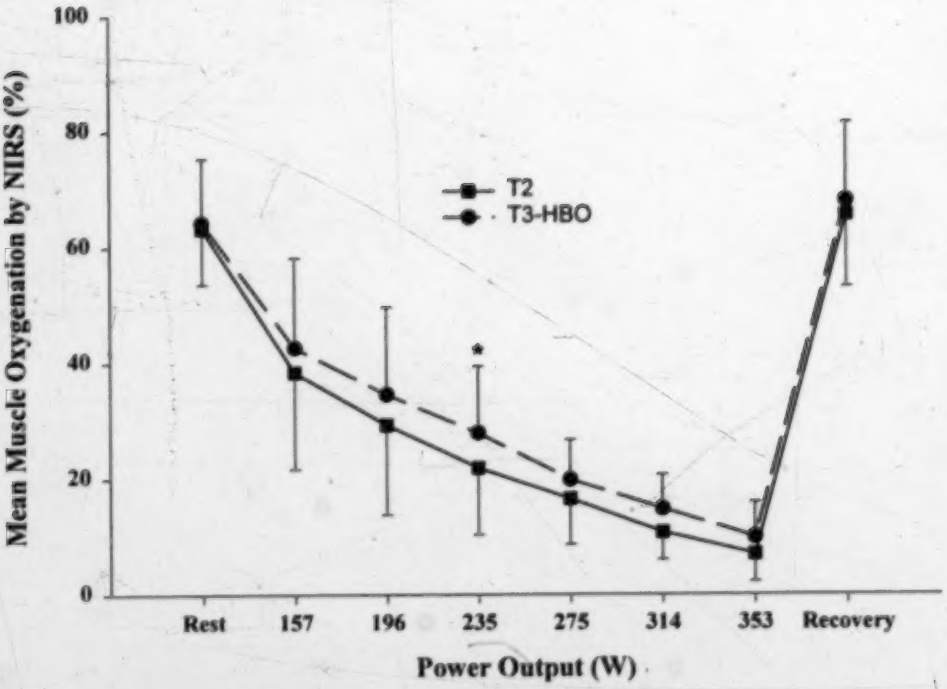


Figure 3.1. Mean muscle oxygenation by near infra-red spectroscopy during rest, individual test workloads and 5 minutes of recovery for T2 (no prior HBO) and T3-HBO (post HBO) [refer to text for a description of the calculation of percent muscle oxygenation].

* T3-HBO significantly greater than T2 ($p < 0.05$). Bars = SD.

CHAPTER FOUR

STUDY 2: EFFECTS OF HYPERBARIC OXYGEN ON RECOVERY FROM EXERCISE-INDUCED MUSCLE DAMAGE

Abstract

There is a paucity of data that supports the use of hyperbaric oxygen (HBO) in sports medicine. The purpose of this study was to determine whether HBO therapy had the ability to accelerate recovery from exercise-induced muscle damage in human gastrocnemius muscle. Twelve healthy male subjects (24.2 ± 3.2 years) performed a strenuous exercise protocol designed to elicit muscle damage and soreness within the right gastrocnemius muscle. Subjects were randomly assigned to either HBO (100% oxygen at 2.5 ATA for 60 minutes; n=6) or sham (atmospheric air at 1.3 ATA for 60 minutes; n=6) treatment conditions. The first treatment was administered 3-4 hours after damage, with a second and third treatment at 24 and 48 hours after the first, respectively. Dependent variables included measures of muscle function using isokinetic dynamometry (peak torque at 0.52 radians·sec^{-1}, peak isometric torque and muscular endurance), muscle cross sectional area using magnetic resonance imaging (MRI), inorganic phosphate and T_2 relaxation time using ^{31}P and ^{1}H magnetic resonance spectroscopy (MRS) respectively and measures of pain sensation and unpleasantness using the Descriptor Differential Scale. These variables were assessed at baseline and until day 5 post-damage. All variables except T_2 relaxation demonstrated statistically significant changes after muscle damage. There was little evidence of a difference in recovery rate between the HBO and sham groups. Faster recovery was observed in the HBO group only for isometric peak torque and pain sensation and unpleasantness. On the basis of the measures employed in the present study, HBO cannot be recommended as an effective method of treatment of muscle injury.

Key words: *Athletic injuries; nuclear magnetic resonance; pain; isokinetic testing; gastrocnemius.*

Introduction

The use of hyperbaric oxygen therapy (HBO) by elite athletes to accelerate recovery from athletic injuries began to gain popularity in the early 1990's (James *et al.*, 1993; James, 1993). HBO is an accepted clinical treatment for a number of conditions that involve low tissue oxygen tensions (Tibbles & Edelsberg, 1996). These conditions include decompression sickness, air embolism and severe carbon monoxide poisoning (Tibbles & Edelsberg, 1996). Athletic injuries typically involve traumatization of soft tissue with accompanying inflammation and the potential for low tissue oxygen levels at the site of the injury (Hunt, 1977; LaVan & Hunt, 1990; James, 1993). Initial interest in HBO in sports medicine was sparked by the proposal that it might have the potential to reduce local hypoxia and inflammation at the site of a soft tissue injury and therefore accelerate the healing process. Other mechanisms that have been proposed to support the role of HBO in sports medicine include its ability to: promote vasoconstriction; quench free-radicals; reduce neutrophil adhesion to blood vessel walls; enhance leukocyte killing; promote collagen synthesis; and promote angiogenesis (neovascularization) of hypoxic spaces (Staples & Clement, 1996).

Numerous professional sports franchises have purchased hyperbaric chambers in the belief that it will help injured athletes return to competition sooner. This has occurred despite the fact that there has been little convincing scientific evidence supporting the use of HBO for sports injuries. The reports that have supported the use of HBO for sports injuries in humans have been mostly anecdotal.

James *et al.* (1993) reported that physiotherapy combined with HBO resulted in a

70% reduction in days lost to injury compared to physiotherapy alone in professional soccer players. However, this study did not have a control group or input from an objective third party (such as a physician) for assessment of injury status. Borromeo *et al.* (1997) investigated the efficacy of HBO in the reduction of morbidity from acute ankle sprains but were unable to find any effects of HBO on the dependent variables measured.

Staples (1999) investigated the ability of intermittent HBO to enhance recovery from exercise-induced muscle injury and soreness in the quadriceps muscle of human subjects. It was found that HBO enhanced the recovery of eccentric strength but had no effect on muscle soreness/pain as measured by a visual analogue scale. Harrison *et al.* (1999) also examined the role of HBO in the treatment of exercise-induced muscle injury of the forearm flexors. Dependent measures in this study included cross sectional area of the involved muscle group and T_2 relaxation time via MRI, isometric strength, perceived soreness and serum creatine kinase. In contrast to Staples (1999), HBO was not found to be effective in speeding recovery from exercise-induced muscle damage as measured by any of these variables.

In animals, the results of studies investigating the role of HBO in recovery from soft tissue injuries have been encouraging. Best *et al.* (1998) examined the use of HBO therapy after an acute muscle stretch injury to the tibialis anterior muscle in rabbits. These investigators found that the animals subjected to HBO possessed a significantly smaller strength deficit between the injured and non-injured limbs and also morphologic studies revealed more complete healing in the treated group seven days after injury. This

suggested that HBO accelerated the recovery after acute muscle stretch injury.

Staples *et al.* (1995) investigated the effects of HBO treatment on the levels of biochemical muscle metabolites in rats after downhill running. They demonstrated that the muscles of rats treated with HBO showed significantly reduced levels of myeloperoxidase - a result suggestive of an inhibitory effect of HBO on the inflammatory process or the ability of HBO to actually modulate the injury to a tissue. Webster *et al.* (1996) demonstrated that exposure of rats to HBO resulted in enhanced recovery of ligament strength and stiffness relative to a group of untreated rats.

Clearly, the use of HBO as an adjunctive therapy in sports medicine is still highly controversial (American Orthopaedic Society for Sports Medicine, 1998; Strauss, 1999). Therefore, the goal of the present research was to further investigate the potential role of HBO in the recovery from soft tissue injury in humans. Specifically, the purpose of this study was to determine if administration of HBO (three treatments of 100% oxygen at 2.5 ATA for 60 minutes) had the ability to accelerate recovery from exercise-induced muscle damage (EIMD) within human gastrocnemius muscle (see Appendix I). It was hypothesized that there would be no effect of HBO on recovery from muscle damage.

Methods

Subjects

Twelve healthy male subjects were recruited from the student body at the University of Alberta (see Appendix C). Table 4.1 contains the subjects' descriptive data.

The sample size was limited to 12 as there were considerable financial and time constraints on the use of the hyperbaric chambers and in-vivo nuclear magnetic resonance facilities.

The individuals who volunteered to participate in the study were required to complete a questionnaire (see Appendix D) indicating that (i) they had never severely injured their right calf muscle, (ii) they were not currently taking or had not recently taken anti-inflammatory medication, (iii) they had not experienced muscle soreness within their right calf muscle during the previous three month period and (iv) that they had not performed weight training or strenuous eccentric training of the lower leg of any kind during the previous three month period. Subjects were also required to complete questionnaires designed to determine if they possessed any contraindications to hyperbaric oxygen therapy and in-vivo nuclear magnetic resonance (see Appendices E and F). Individuals that were within the age range of 18 to 30 years and who indicated "no" or "true" to all of the questions posed in the questionnaires were deemed to be appropriate subjects for participation in the study.

Ethical approval for this study was obtained from the Health Research Ethics Approval Board of the University of Alberta Hospital, Edmonton. All subjects were fully informed as to the study procedures and time commitment involved (the participant information letter is contained in Appendix G) and each was required to sign an informed consent form indicating that they understood the study procedures and the risks and benefits of participation (see Appendix H).

Experimental design

The time course design of the study is shown in Figure 4.1. The timing of the tests was consistent between days to minimize the effects of potential daily circadian variations on the measured variables. On the two days prior to undergoing the exercise protocol designed to elicit muscle damage and soreness, every subject was required to complete two isokinetic testing sessions designed to assess the baseline strength and endurance of the plantar flexor muscles of the right lower leg. Subjects were also oriented to the pain questionnaire (the Descriptor Differential Scale) on the day preceding the damage protocol in the manner described below. Finally, on the day prior to the damage protocol, subjects completed one baseline session of magnetic resonance imaging (MRI) and spectroscopy (MRS) (detailed below).

Within 3-4 hours of the damage protocol, subjects underwent the first session of either hyperbaric oxygen (60 minutes of 100% oxygen at 2.5 ATA) or sham conditions (60 minutes of air at 1.3 ATA). Subjects underwent a second and a third session within the hyperbaric chambers exactly 24 and 48 hours after the first.

MRI/MRS testing was repeated on the first, third and fifth day after the damage protocol at a similar time of the day to the baseline MRI/MRS test. Isokinetic muscular strength and endurance testing was repeated (also at similar times of the day) on the first, second, third and fifth day after the damage protocol. Prior to every post-damage isokinetic strength test, subjects were required to complete the Descriptor Differential Scale that assessed the intensity and unpleasantness of the pain/soreness that they were

experiencing.

Exercise/damage procedure

The exercise protocol designed to elicit muscle damage was performed using both legs on a standing Universal calf raise machine within the Fitness and Lifestyle Centre at the University of Alberta (see figure 4.2). The gastrocnemius was selected as the muscle for investigation in this study as it is a convenient muscle group to study within the narrow confines of the magnet bore used for MRI/MRS. Even though the right gastrocnemius only was monitored for recovery, the exercise protocol was performed with both calves. Subjects were first instructed to perform a warm up set of 10 complete repetitions with approximately 113.6 kg (250 pounds). Athletic tape was placed on the footrest of the machine (a) to prevent slipping of the feet during the exercise procedure and (b) to mark and standardize the position of the feet during each set performed (the feet were placed at slightly less than shoulder width apart). A number of points were reinforced to the subjects during the warm-up including: (i) relax the upper body and shoulders as much as possible during all repetitions, (ii) perform all repetitions through a full range of motion at a slow controlled speed, (iii) the knees must be kept straight during all repetitions with no bouncing of the load at any stage of the movement and (iv) the body must be supported by the balls of the feet on the footrest of the machine.

In order to standardize the range of motion over which subjects were working, the following procedure was followed for each individual subject. After the warm-up had

been completed, subjects were requested to stand and relax within the machine with the knees fully extended and the 113.6 kg supported by the shoulder pads. A mark was made on the machine which corresponded to this starting point in the range of motion. The subject was then requested to plantarflex maximally and another mark was made on the machine corresponding to the end point in the range of motion. The subject was then requested to step off the machine and the distance between the two points, the total range of motion, was measured. The point half way between these marks was noted and was used to define the failure point in the range of motion (described below).

In order to determine the weight to be used for the damage procedure (80% of 1-repetition maximum {1-RM}), it was first necessary to determine the 1-RM for each subject. This was done immediately prior to the damage protocol itself as this avoided the possibility of the 1-RM procedure effectively "pre-adapting" the muscles against subsequent muscle damage (Clarkson *et al.*, 1992; Ebbeling & Clarkson, 1989; Ebbeling & Clarkson, 1990; Clarkson & Tremblay, 1988; Nosaka *et al.*, 1991; Mair *et al.*, 1995). Two minutes after the warm-up had been completed, subjects started the 1-RM testing procedure by completing one set of 10 repetitions using a load between 159.1 kg (350 lbs) and 181.8 kg (400 lbs). Weight was gradually added to subsequent test sets until 1-RM for each subject was determined. One repetition maximum was defined as the load that the subject could lift only once through the pre-determined full range of motion. A two minute rest was permitted between sets and all subjects completed the 1-RM test protocol within five sets.

The load used for the damage protocol was 80% of 1-RM. Each subject

performed five sets of calf raises (both concentric and eccentric actions) to failure with a two minute recovery period between sets. Failure was defined as the inability to reach the mid-range point for two consecutive repetitions. Subjects were encouraged to exert maximal effort during all sets with good form.

Hyperbaric oxygen therapy:

Prior to the first entry into the hyperbaric chamber, subjects were given a thorough medical examination by a physician to ensure that they possessed no contra-indications to hyperbaric therapy. Prior to every subsequent hyperbaric treatment, subjects were questioned by the hyperbaric technicians concerning a change in their health status (such as a cold or sinus problem) that may have caused difficulty during compression or decompression. All subjects' eardrums were inspected before and after every hyperbaric treatment to ensure that no pathology was evident. One subject did indicate a history of ear surgery and special care was taken to ensure that this subject did not experience problems during hyperbaric therapy. For this subject, compression rate was reduced and the subject was provided with ear plugs designed to reduce the pressure differential across the eardrum during descent (compression). HBO or sham treatments were performed in a Sechrist monoplace hyperbaric chamber (Sechrist Industries Inc., Anaheim, CA.) under the supervision of trained medical personnel at the Misericordia Hospital, Edmonton (see figure 4.3). Six subjects were assigned to HBO and six were assigned to the sham treatments by random selection in a single blind manner. The gas

supply to the chambers was covered with drapes to ensure that subjects were blinded to the treatment that they were receiving.

For the HBO treatments, subjects were compressed to 2.5 ATA (253 kPa) over a period of 10-20 minutes whereupon they were exposed to free 100% O_2 for a duration of 60 minutes. At the end of the 60 minute period, 5-10 minutes were allowed for decompression to atmospheric pressure. The pressure of 2.5 ATA and time of 60 minutes was selected for two reasons. First, the partial pressure of O_2 at 2.5 ATA (approximately 1840mmHg) posed little risk of toxicity to the subjects, especially with a treatment time of 60 minutes. Second, previous research that had failed to find an effect of HBO in a sports medicine setting had used 2.0 ATA as the treatment pressure (Staples, 1999; Borromeo *et al.*, 1997; Webster *et al.*, 1998).

For the sham (placebo) treatments, subjects were compressed to 1.3 ATA (approximately 132kPa) and breathed room air for 60 minutes at this pressure. This pressure was sufficient to ensure that subjects experienced the symptoms of external pressure changes but it did not result in marked increases in oxygen tension experienced by the subjects (the partial pressure of oxygen within the chamber under these conditions was approximately 195mmHg). Aside from this difference, the protocol for the sham and HBO treatments was identical to reduce the likelihood that subjects were aware of their assigned treatment groups.

Staples & Clement (1996) suggested that HBO should be administered within hours after the initial injury for HBO to be maximally effective. Therefore, HBO or sham treatments were administered to each subject between 3-4 hours after the damage

protocol, when acute inflammatory responses of muscle after exercise-induced damage first become evident (Armstrong *et al.*, 1991; Smith, 1991; Pyne, 1994; Tidball, 1995). The treatments were repeated at exactly the same time of day on the two subsequent days (approximately 27-28 and 51-52 hours post-damage) during which time the inflammatory processes associated with exercise-induced muscle damage have been found to be most evident within muscle (Armstrong *et al.*, 1991; Smith, 1991; Pyne, 1994; Tidball, 1995; Takahashi *et al.*, 1994). Each subject received either three HBO treatments or three sham treatments.

Determination of muscular strength and endurance via isokinetic dynamometry:

On two separate occasions prior to performing the damage protocol and on four occasions after (see Figure 4.1), subjects underwent functional strength and endurance testing of the right plantarflexors on a Cybex 340 isokinetic dynamometer (Lumex Inc., New York) that was calibrated prior to every testing session. Two pre-tests were performed due to the variability associated with isokinetic muscle testing (Dvir, 1995). The highest value recorded during the isokinetic pre-tests was taken as the baseline measure for each variable.

Subjects were positioned supine with the right leg fully extended for the isokinetic testing (see Figure 4.4) and the right foot placed on a Cybex foot plate and secured tightly with velcro straps. The left knee was flexed to 90 degrees and the left foot placed on a footrest. Subjects were secured with straps around the hips and right thigh to minimize

excessive body motion and subjects were required to cross the arms over the chest during all testing procedures (see figure 4.4). Care was taken to note the precise position of the foot for each individual and this was replicated for all subsequent isokinetic testing (Andersen, 1996). Alignment of the ankle with the axis of rotation of the isokinetic device was carried out with respect to an imaginary axis connecting the medial and lateral malleoli as suggested by Dvir (1995).

Once the subject was positioned correctly, a warm up was performed. This consisted of 30 submaximal repetitions at 1.04 radians·sec^{-1} (60 degrees·sec^{-1}) through a full range of motion followed by 90 seconds of passive rest and then 10 submaximal repetitions at 0.52 radians·sec^{-1} (30 degrees·sec^{-1}). Two minutes of passive rest were then provided. Peak torque was determined during concentric plantar flexion exercise at 0.52 radians·sec^{-1}. Subjects were requested to perform 4 plantarflexion repetitions as hard and as fast as possible and to ensure that maximal dorsiflexion occurred prior to each repetition. This procedure was repeated after 90 seconds of rest. The highest peak torque attained during the two test sets was recorded.

After two minutes of passive rest, the position of the ankle joint was fixed at 0 degrees with the aid of a goniometer and two test sets were performed to measure isometric muscle strength. The two isometric sets were separated by 90 seconds of passive rest. Subjects were requested to push as hard as possible against the foot plate using only the plantar flexor muscle group. The torque being generated by the subject was visible to the investigator on a computer screen and subjects were encouraged to continue to push until it was apparent that torque had reached a maximum. At this time

they were signalled to stop. The highest torque obtained during either of the two sets was recorded.

After a further two minutes of passive rest, the muscular endurance of the plantar flexors was assessed using a protocol that consisted of 20 maximal plantarflexion contractions through a full range of motion at a speed of 1.04 radians·sec^{-1} (60 degrees·sec^{-1}). The total work performed during the set was measured. It has been determined previously that absolute measures of muscular endurance, such as total work performed, demonstrate greater reliability and lower variability than relative measures of muscular endurance (Montgomery *et al.*, 1989; Burdett & Van Swearingen, 1987).

In-Vivo Nuclear Magnetic Resonance procedures:

Prior to the first MRI/MRS session, the maximum circumference of the right calf muscle was determined for each subject. Subjects were then required to lie supine on the magnet bed and position their right lower leg such that the maximum circumference of the lower leg was directly over the centre of a surface coil that was used for ^{31}P MRS. With the aid of laser positioning, cross hairs were marked on the subjects' shins with semi-permanent ink to ensure that images and spectra were collected at the same site at each testing session. Subjects were informed to avoid erasing these marks during the period of the study. Once correct positioning had been achieved, the lower leg was secured in position with the aid of towels and velcro straps.

Experimental NMR data were acquired in a 3T whole-body scanner (magnet from

Magnex Scientific PCL, console from Surrey Medical Imaging Systems Ltd.). All ^{1}H measurements were done using a quadrature birdcage resonator for radio frequency transmission and signal reception. Multi-slice gradient echo imaging in the transverse, sagittal and coronal planes were used to register the STEAM (stimulated echo acquisition mode localization) selected volume precisely to the medial gastrocnemius muscle where most evidence of soreness and damage occurred. For measurement of ^{1}H T_2 relaxation time, a series of spectra were acquired at rest on subjects using a STEAM pulse sequence to determine the TE dependence of the water peak (TR = 6s, TE = 20 - 220 ms [40 time points in 5 ms increments], 2048 data points, 4 averages).

The ^{31}P magnetic resonance spectra (see figure 4.5) were acquired using an 8 cm diameter surface coil, and with 1D-image selected *in-vivo* spectroscopy (ISIS) localisation (Ordidge *et al.*, 1986). To maximise the signal from the medial gastrocnemius muscle, the 1D slice was co-registered with the STEAM localised volume with the most distant edge of the slice bisecting the interface between the medial and lateral gastrocnemius muscles (Figure 4.6).

Following zero-filling (to 4096 data points), filtering (^{1}H data with a 2Hz exponential, and ^{31}P data with a 8 Hz exponential), and fast Fourier transformation of the time domain data, the peak areas were estimated using the PERCH analysis package, where each peak was fitted to a Gaussian lineshape. The water peak area from the ^{1}H data was used for T_2 estimation using a non-negative-least-squares (NNLS) algorithm for multi-exponential fitting that requires no *a priori* assumptions of the number of decaying components. In addition, the decay curves were fitted to a single exponential in order to

allow a comparison of the T_2 measurement used in the present study to those used in previous studies that have investigated the course of recovery from muscle damage (Mair *et al.*, 1992; Nosaka & Clarkson, 1996; Shellock *et al.*, 1991; Takahashi *et al.*, 1994; Rodenburg *et al.*, 1994; Harrison *et al.*, 1999; Sorichter *et al.*, 1995; Nurenburg *et al.*, 1992). The single exponential fit gave a weighted average of all the T_2 decay components within the muscle.

After estimation of the ^{31}P spectral peak areas, the inorganic phosphate concentration (P_i) was calculated as a percentage of the total combined areas under the P_i and PCr peaks ([P_i/ P_i + PCr] * 100). This percentage value is a reflection of the state of the following equilibrium reaction catalyzed by creatine kinase: $PCr \leftrightarrow Cr + P_i$. A greater percentage of P_i indicates that the reaction equilibrium has been pushed further to the right, which in turn implies a greater rate of turnover of PCr and ATP within the muscle.

The above MRI/MRS procedures were done on the day prior to damaging the muscle, and then repeated on days 1, 3 and 5 post-damage.

Determination of cross sectional area of the gastrocnemius muscle:

Three adjacent transverse gradient echo images (TR = 500 ms, TE = 22 ms, slice thickness = 3 mm, centre to centre = 5 mm) were used to calculate the cross sectional area of the medial gastrocnemius muscle. The middle of the three images corresponded to the maximum circumference of the lower leg. The contour of the medial gastrocnemius

muscle was traced with a cursor and the area of the outlined muscle (in cm^2) was determined using Image-Pro Plus software (Media Cybernetics, MD). The exact dimensions of the images imported into the software were known and were used for measurement calibration. The mean value of the three adjacent images was taken as the final cross sectional area of the muscle.

The same procedure as above was followed to determine the cross sectional area of the bone marrow within the tibia. This area was unchanged throughout the course of the study and therefore served as a standard against which changes in muscle cross sectional area could be compared.

Measurement of pain and soreness:

The intensity and unpleasantness of delayed onset muscular pain/soreness was assessed immediately prior to every isokinetic strength test by means of the Descriptor Differential Scale (DDS) (Gracely & Kwilosz, 1988). The DDS has been validated (Doctor *et al.*, 1995) and found to satisfy standard psychometric criteria for reliability, objectivity and item homogeneity (Gracely & Kwilosz, 1988). The DDS consists of two forms - the sensory-intensity form (Figure 4.7) is designed to assess the intensity of pain sensation and the unpleasantness form (Figure 4.8) is designed to ascertain how the subjects interpret this pain sensation. Each form has twelve descriptor items that are randomly ordered each time a subject completes it (to prevent recall of previous responses). Each descriptor item is centred over 21 horizontal dashes with a minus sign

('-') over the far left dash and a plus sign ('+') over the far right dash. Subjects indicate their pain in relation to each descriptor by checking a single dash in relation to the descriptor. A check on the dash under the descriptor indicates pain sensation equal to the intensity implied by the descriptor, a check on a dash to the left indicates pain intensity proportionally less than that implied by the descriptor, a check to the right indicates pain intensity proportionally more intense (Gracely & Kwilosz, 1988).

Subjects were oriented to the DDS by the principal investigator prior to experiencing muscle soreness. In order to ascertain that subjects fully understood the correct manner in which they should complete this scale, they were provided with an imaginary painful scenario (a pin prick) and were asked to complete the two DDS forms with this scenario in mind. When the subject had completed this task, their responses on each of the items was arranged in order of increasing magnitude to ascertain if the responses were consistent (described by Gracely & Kwilosz, 1988). All subjects achieved acceptable consistency after the orientation session.

In order to assess the level of discomfort within the gastrocnemius muscle on the days subsequent to the damage protocol, subjects were requested to contract the right calf muscle while standing (*ie.* stand on "tip-toes") and then to rate the level of pain and unpleasantness using the two DDS forms. Each subject completed the DDS on a total of four occasions post damage.

In order to analyze the pain data quantitatively, the dashes under each descriptor term were given a value from 0 (far left dash) to 20 (far right dash). The total for all 12 descriptor terms was determined for both forms. Therefore, there was a theoretical

"maximum" achievable pain and/or unpleasantness score of 12 x 20 = 240 (arbitrary units).

Statistical analysis:

Means and standard deviations are reported and data were analyzed using a univariate analysis of variance (ANOVA). A two way (group x time) ANOVA was performed with repeated measures on each dependent variable. Multiple comparison procedures for significant F ratios were performed using a Newman-Keuls analysis. Alpha was set *a priori* at $p<0.05$. The statistical software package used was Statistica (Oklahoma City).

Results

As shown within Table 4.1, the subjects within the HBO group were, on average, heavier individuals with concomitantly larger and stronger calf muscles. However, these differences did not attain statistical significance.

Tables 4.2, 4.3 and 4.4 show the absolute peak torque values obtained over the course of the study at 0.52 radians·sec^{-1}, peak isometric torque and muscular endurance respectively. All demonstrated significant main effects over both time and group but no interactions were detected. Figures 4.9 to 4.11 illustrate the percentage change (relative

to the baseline measure) in peak torque at 0.52 radians·sec^{-1}, peak isometric torque and muscular endurance respectively. Statistically significant main effects for time were seen in all of these figures. There was a significant interaction effect of group and time on isometric peak torque only (Figure 4.10). In this case, there was a significant decrease in isometric peak torque from baseline to days 1 and 2 in the sham group but not in the HBO group.

Table 4.5 shows the ratio of (P_i/P_i + PCr) * 100 (the area under the P_i peak expressed as a percentage of the total combined areas under the P_i and PCr peaks). A statistically significant main effect of the damage was evident over time with the largest increase in P_i evident the day after muscle damage occurred. However, there was no statistically significant difference in the rate of recovery between groups. Figure 4.12 illustrates the percentage change in (P_i/P_i + PCr) * 100 relative to the baseline measure. Again, a main effect of the damage is clearly evident but there were no differences in recovery rate between groups.

Table 4.6 provides both individual and group mean T_2 data determined with both multi-exponential NNLS fitting and also mono-exponential fitting. The separated T_2 decay components determined from the NNLS technique are shown. Examination of the NNLS data reveals that there is always a short T_2 component between 20 and 40 milliseconds. However, there is little reproducibility in the appearance of a longer component, both within subjects and from one MRI/MRS study to the next in the series. For this reason, only the shorter component (representative of the intracellular water compartment) was used to calculate the mean T_2 values for each group. No statistically

significant differences were detected in the estimated T_2 values, when comparing the pre-damage to post-damage studies, whether NNLS or mono-exponential fitting was employed. Figures 4.13 and 4.14 illustrate the mean percentage changes for each group from baseline when using NNLS and mono-exponential fitting respectively. There was no evidence of a statistically significant change in these variables after muscle damage.

Tables 4.7 and 4.8 show the absolute cross sectional area measured at each of the four MRI/MRS sessions for the medial gastrocnemius and tibial bone marrow. No statistically significant changes were seen in the bone marrow area but a main effect of damage on the muscle area was detected. No significant difference in recovery rate of muscle cross sectional area was observed between the groups. Figure 4.15 illustrates the percentage change in medial gastrocnemius cross sectional area over time relative to baseline. The main effect of damage on the muscle area is clearly evident from pre to day 1 post-damage but there were no differences between the groups.

Tables 4.9 and 4.10 reveal the absolute values of pain sensation and unpleasantness reported over time. For both these data sets, an interaction effect was observed. Both pain sensation and unpleasantness rose to a maximum on day 2 post-damage. From this time point, there was a more rapid decline in both variables in the HBO group until day 5 post-damage. On day 5, the HBO group reported significantly less pain sensation and unpleasantness than the sham group. Figures 4.16 and 4.17 illustrate the changes that occurred in pain sensation and unpleasantness during the course of the study. The values in these figures were calculated by expressing the absolute score as a percentage of the maximum score reported by that subject. There was a significant

increase in both groups in the pain and unpleasantness experienced as a result of muscle damage. For both groups, these both peaked on day 2 post-damage and then resolved over the subsequent days. The more rapid recovery from pain and unpleasantness in the HBO group is apparent, with this group reporting significantly less of both variables on day 5 post-damage.

Discussion

There is very little evidence in the literature to support the use of HBO therapy in sports medicine. This study was designed to investigate the effects of HBO (three treatments of 100% oxygen at 2.5 ATA for 60 minutes) on recovery from exercise-induced muscle damage within human gastrocnemius muscle.

Exercise/damage protocol:

It is well known that the basic mechanisms underlying the response of muscle to acute trauma vary little, regardless of the nature of the initial insult to muscle (Tidball, 1995). Therefore, muscle damage induced by high force lengthening contractions is a useful model that can be used to investigate the effect of HBO on recovery from muscular injury sustained as a result of exercise. It is an ethically acceptable manner of inducing muscle damage in human subjects, being fully repairable and having no lasting

detrimental effects (Clarkson *et al.*, 1992). The exercise protocol employed in this study to elicit exercise-induced muscle damage (EIMD) was unlike those used in most previous studies (Nosaka *et al.*, 1991; Cleak & Eston, 1992; Clarkson & Tremblay, 1988; Staples, 1999; Rodenburg *et al.*, 1994; Harrison et al., 1999; Mair *et al.*, 1992; Mair *et al.*, 1995; Sorichter *et al.*, 1995; Takahashi *et al.*, 1994; Nosaka & Clarkson, 1996) in that it contained a substantial concentric as well as an eccentric component. Pilot work was performed to determine if it was possible to perform a purely eccentric plantar flexion procedure. However, it was decided for reasons of safety and comfort for the subjects to proceed with a normal standing calf raise movement utilizing both shortening (concentric) and lengthening (eccentric) contractions of the triceps surae muscle group.

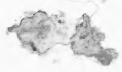
The exercise protocol did have a significant effect on all of the dependent variables measured except for T_2 relaxation time as measured by in-vivo nuclear magnetic resonance. Therefore, the protocol employed was successful in eliciting biochemical, functional and sensory evidence of damage within the gastrocnemius muscle.

An advantage of utilizing EIMD as a model of muscle injury is that the quantity of injury within a muscle can be at least partially standardized. All subjects within the present study were required to complete an exercise protocol that was adjusted relative to their individual strength level. It would not be accurate to suggest that this resulted in similar quantities of damage within the muscles of different subjects. However, it would be reasonable to say that there should be less variability in the amount of injury between subjects than one might expect from investigation of other forms of soft tissue "sports" injuries. Clearly, the quality (or type) of damage experienced by the subjects should be

similar as a result of the exercise protocol used.

Conversely, a disadvantage of EIMD is that its etiology is distinct from other forms of sports injuries, such as those involving joint pathology (ankle or knee sprains for example). Therefore, it may not be possible to extrapolate results to other kinds of soft tissue "sports" injuries.

Isokinetic muscular strength and endurance data:



For all absolute isokinetic measures, there were significant differences at every time point between groups. This finding was most likely a reflection of the fact that the HBO group possessed, on average, larger and stronger calf muscles (table 4.1). This discrepancy occurred because matching of subjects for size and strength prior to the start of the study was not possible due to subjects being recruited while the study was in progress.

When collapsed across groups, all absolute isokinetic measures taken in the present study demonstrated significant decreases after muscle damage had been elicited. The greatest decrements were observed approximately 24 hours after the damage occurred, a finding that agreed with previous work (Clarkson *et al.*, 1992; Nosaka *et al.*, 1991; Cleak & Eston, 1992). Recovery to baseline strength and endurance levels was complete after five days in both groups in the present study. This was somewhat faster than has been documented in most previous research and may have been related to the population and muscle group used in the present study and/or the protocol used to elicit

muscle damage.

The subjects in the present study had not performed strenuous weight training of the calf muscles for three months prior to the study but many of them were athletes who were engaged in other activities requiring strenuous use of the legs. Therefore, one would expect the gastrocnemius to be at least partially pre-adapted to muscle damage which would result in less damage produced after eccentric exercise and hence a faster recovery to baseline muscular performance levels (Clarkson *et al.*, 1992; Nosaka *et al.*, 1991; Cleak & Eston, 1992). Most of the above studies in this area have investigated muscle groups (usually either the forearm flexors or the quadriceps) in sedentary subjects and therefore there was potential for greater damage to occur on performance of unaccustomed eccentric exercise.

As mentioned above, the exercise protocol used in the present study was not typical of that used in most previous investigations of exercise-induced muscle damage. Most studies (Nosaka *et al.*, 1991; Cleak & Eston, 1992; Clarkson & Tremblay, 1988; Staples, 1999; Rodenburg *et al.*, 1994; Harrison et al., 1999; Mair *et al.*, 1992; Mair *et al.*, 1995; Sorichter *et al.*, 1995; Takahashi *et al.*, 1994; Nosaka & Clarkson, 1996) have employed purely eccentric muscle contractions to elicit damage. The present study used an exercise protocol that consisted of both eccentric and concentric contractions. The concentric actions may have caused the gastrocnemius muscle to fatigue prematurely, thus preventing the performance of further eccentric work and reducing the amount of damage sustained by the muscle.

With the exception of peak isometric torque, there was no effect of HBO on

recovery of isokinetic muscular performance. There was a significant percentage decrease in isokinetic peak torque at 0.52 radians·sec^{-1} after muscle damage in both groups that was still statistically different from baseline at day 3 post-damage. There was no evidence of an attenuated loss in strength or faster recovery in the HBO group. A similar pattern was observed for the percentage change in muscular endurance. Most importantly, the loss in muscular endurance post-damage and the recovery rate was not different between groups. Therefore, HBO did not appear to enhance recovery of either isokinetic muscular peak torque at 0.52 radians·sec^{-1} or isokinetic muscular endurance.

In contrast, a significant interaction of group and time was observed for the percentage change in peak isometric torque. After muscle damage, the sham group experienced a significant decrease in isometric peak torque that was still significantly different from baseline at day 2. The HBO group, however, experienced only a slight, non-significant decrease in isometric torque. Therefore, HBO appeared to confer a protective effect against the loss of isometric strength after muscle damage. If one assumes that this was a real effect (and not an artefact due to small group size), the physiological mechanism is difficult to explain, especially in view of the fact that there was no effect of HBO on peak isokinetic torque at 0.52 radians·sec^{-1} and isokinetic muscular endurance.

A possible explanation for the above result may involve the mechanisms responsible for maintaining calcium homeostasis within peripheral skeletal muscle. There is much evidence to suggest that exercise-induced muscle damage causes sarcoplasmic reticular (SR) structural changes and dysfunction within muscle cells (Byrd,

1992). Scherer & Deamer (1986) determined that one mechanism by which muscle damage impairs the function of SR is through free radical-mediated oxidation of sulphydryl groups within the SR Ca^{2+}-ATPase pump. This results in a loss of calcium homeostasis within the muscle cells and thus a reduced ability to generate tension. HBO has been shown to have the ability to decrease evidence of free radical-mediated damage within skeletal muscle by preventing excess neutrophils from adhering to vessel walls at the site of an injury and initiating a respiratory burst (Zamboni *et al.*, 1993). Therefore, HBO treatment might have resulted in reduced free radical-mediated damage of the SR Ca^{2+}-ATPase pump that was reflected by improved subsequent isometric muscular performance. It is feasible that this effect was only large enough (and therefore statistically detectable) for the maximal voluntary isometric contractions where muscle fibre recruitment was at its greatest.

A further potential explanation of the protective effect of HBO on loss of isometric peak torque is that the subjects within the HBO group were better able to recruit muscle fibres for the isometric contraction during the two days subsequent to muscle damage (*ie*. a central as opposed to a peripheral effect). However, the involvement of the central nervous system is difficult to rationalize, particularly in view of the fact that reported pain sensation and unpleasantness were not different between groups on days 1 and 2.

It is also possible, though unlikely, that the different patterns of recovery of isometric torque between the groups may have been related not to the HBO treatments but to the fact that the HBO group had, on average, larger calf muscles than the sham

group. However, it is difficult to rationalize how absolute size and/or strength of the calf muscles might influence the loss and/or recovery of isometric torque after EIMD.

Two other studies in the literature have investigated the effects of HBO on recovery from exercise-induced muscle damage (Staples, 1999; Harrison *et al.*, 1999). Staples (1999) found that subjects treated with intermittent HBO (100% oxygen for 60 minutes at 2 ATA) over three and five days had augmented recovery of eccentric strength when compared to subjects receiving sham conditions. The precise mechanism responsible for this effect was not clear. Harrison *et al.* (1999) employed very similar experimental methodology to the present study to investigate the effects of HBO on recovery from muscle damage within the forearm flexors of human subjects. Despite a substantially longer total exposure time to HBO (500 minutes) than that employed in the present study and by Staples (1999), no effect of the treatment was found. As no measurements of recovery were taken between 24 hours and 7 days post-damage, it is possible that Harrison *et al.* (1999) may have missed detection of a positive effect of HBO.

For obvious reasons, athletes typically employ a calculated and cautious approach when rehabilitating an injured body part. A reasonable concern could be raised that the strenuous isokinetic testing procedure employed during the present study was not representative of a typical rehabilitation protocol and may have exacerbated damage or compromized the healing process within the muscle. This was unlikely, however, as the isokinetic testing procedures were concentric in nature, which posed less threat of mechanical disruption to muscle than eccentric actions (Armstrong *et al.*, 1991).

To summarize, HBO did not have any effect on the recovery of isokinetic peak torque at 0.52 radians·sec^{-1} or isokinetic muscular endurance after exercise-induced muscle damage. However, HBO did appear to confer a protective effect against the loss of isometric strength after muscle damage.

In-Vivo Nuclear Magnetic Resonance data:

Several studies using ^{31}P-MRS (Aldridge *et al.*, 1986; McCully *et al.*, 1988; Rodenburg *et al.*, 1994; Rodenburg *et al.*, 1995) have shown a significant increase in the inorganic phosphate peak (P_i) following damaging eccentric exercise that typically peaks approximately 24 hours post-exercise and may remain elevated for up to 10 days post-exercise (see Appendix I). This observation was confirmed in the present study with recovery being incomplete at 5 days post-damage. No statistically significant difference between the HBO and sham groups was found at any time and therefore it appeared that HBO did not have an effect upon either the magnitude of the biochemical disruption within the muscle or on the rate of tissue recovery.

The precise causes of raised P_i within eccentrically damaged muscles remain unclear. A number of possible mechanisms have been proposed including increased activity of ion transport pumps to compensate for "leaky" membranes, alterations in mitochondrial function and an influx of P_i-rich extracellular fluid into damaged muscle cells (McCully *et al.*, 1988; Aldridge *et al.*, 1986). The latter mechanism would be expected to result in the concomitant appearance of a longer T_2 component within skeletal

muscle (discussed further below) – a phenomenon that was not found in the present study. The most widely accepted explanation of raised intracellular P_i following eccentric exercise is an increase in overall resting cell metabolism owing to repair processes such as increased protein synthesis (Rodenburg *et al.*, 1994; McCully *et al.*, 1988; McCully & Posner, 1992). If the latter explanation is correct, a desirable effect of HBO might be reflected by an increase in the amount of P_i relative to the sham group during the day(s) subsequent to muscle injury. However, this was not observed in the present study.

Saab *et al.* (1999) have reported that there are four T_2 relaxation components within in vivo skeletal muscle, with time constants at <5, 21 ± 4, 39 ± 6 and 114 ± 31 milliseconds and relative proportions of 11 ± 2, 28 ± 15, 46 ± 12 and 11 ± 5% respectively. These investigators suggested that the shortest and longest components corresponded to water associated with macromolecules and extracellular water, respectively. The middle T_2 components (that contributed the majority of the total decay signal) were attributed to intracellular water. In the present study, a maximum of two T_2 decay components was detected due to differences in the data acquisition protocol. A large component between 20 and 40 milliseconds, most likely due to intracellular water, was always detected. However, there was no consistency in the appearance of a longer component and when one was apparent, its value and its proportional contribution to the total decay signal was highly variable. For this reason, only the shorter component was used for statistical analysis. This limited the T_2 investigation to the intracellular compartment and yielded a mean (SD) NNLS T_2 relaxation time for the 12 subjects in the

present study prior to muscle damage of 31.3 (3.7) milliseconds.

Several studies (Mair *et al.*, 1992; Nosaka & Clarkson, 1996; Shellock *et al.*, 1991; Takahashi *et al.*, 1994; Rodenburg *et al.*, 1994; Harrison *et al.*, 1999; Sorichter *et al.*, 1995; Nurenburg *et al.*, 1992) have reported that after exercise-induced muscle damage, the overall T_2 relaxation time of skeletal muscle becomes prolonged, a finding attributed to a greater accumulation of extracellular water (oedema) within the muscle (see Appendix I). All these studies measured T_2 using a T_2-weighted spin-echo imaging sequence and increases over baseline measures were found to range from approximately 10% to 80%, peaking anywhere from 1 day to 7 days post-damage. The longer T_2 has been found to remain, in some cases, for up to 80 days post-damage in some subjects (Shellock *et al.*, 1991).

In the present study there was no evidence of a significantly longer post-damage T_2. Furthermore, no differences were seen between groups at any time. If damage had caused appreciable extracellular oedema to develop within the muscle, the appearance of a consistent long T_2 decay component would have been expected (Saab *et al.*, 1999). Alternatively, if appreciable intracellular oedema or an increase in the intracellular free space had developed, an increase in the short T_2 component(s) would have been expected. Neither of these phenomena were observed.

The reason for the lack of an effect of muscle damage on T_2 in this study is not clear. It is possible that the studies that reported an increase in T_2 (Mair *et al.*, 1992; Nosaka & Clarkson, 1996; Shellock *et al.*, 1991; Takahashi *et al.*, 1994; Rodenburg *et al.*, 1994; Harrison *et al.*, 1999; Sorichter *et al.*, 1995; Nurenburg *et al.*, 1992) elicited

substantially more damage within skeletal muscle than occurred in the present study. As discussed above, the calf muscles of some of the subjects in this study may have been partially pre-adapted to muscle damage which would have resulted in less damage produced after eccentric exercise. Most previous studies have used sedentary subjects whose muscles may have been more susceptible to damage.

A further potential explanation for the lack of a change in T_2 is the manner in which it was measured in the present study. Studies that have detected an increase in T_2 as a result of muscle damage (Mair *et al.*, 1992; Nosaka & Clarkson, 1996; Shellock *et al.*, 1991; Takahashi *et al.*, 1994; Rodenburg *et al.*, 1994; Harrison *et al.*, 1999; Sorichter *et al.*, 1995; Nurenburg *et al.*, 1992) have calculated it from a T_2-weighted spin-echo imaging sequence. This method of measurement ignores the fact that there are multiple separate T_2 components within skeletal muscle (Saab *et al.*, 1999) and provides only a weighted average of these components. This implies, incorrectly, that T_2 decay within muscle is mono-exponential. However, to verify that the technique of measurement employed in the present study was not the cause of the unchanged T_2, the data was reprocessed to yield single mono-exponential T_2 values. Again, no significant differences were detected either between groups or over time.

A final possibility for the lack of statistical change in T_2 is the small sample size employed in this study. Examination of the changes in T_2 for the combined groups from baseline to day 1 post-damage reveals that the mean increase in NNLS T_2 was 7.0% and that of the mono-exponential T_2 was 6.6%. These changes may be of physiological significance but evidence of a statistical difference may have been prevented by the

combination of a small sample size and the inherent variability of T_2 measures within skeletal muscle.

An increase in the cross sectional area of the medial gastrocnemius muscle was detected in both groups in this study (see figure 4.15) and was most evident the day after muscle damage was elicited. As expected, the cross sectional area of the tibial bone marrow was unchanged during the course of the study which confirmed that the change in area of the muscle was a real change and not systematic measurement error. There were no differences between the groups in either the magnitude of the increase in cross sectional area or the rate at which recovery towards the baseline occurred. Therefore, HBO was not effective in preventing the "swelling" of the muscle or in enhancing recovery from this swelling.

When the data from both groups were combined, the mean increase in area of the medial gastrocnemius muscle on day 1 was 5.4% relative to the baseline measure. This was similar to the 6% increase found in the quadriceps muscle by Takahashi *et al.* (1994) but less than the 13% increase found by Rodenburg *et al.* (1994) and the 21% increase found by Harrison *et al.* (1999). The time course of this change in cross sectional area of the damaged muscle in the present study was similar to that reported by Takahashi *et al.* (1994) and Harrison *et al.* (1999). These investigators detected the greatest increase in muscle cross sectional area at approximately 24 hours post-damage with a gradual return to baseline over subsequent days. Rodenburg *et al.* (1994), however, detected the greatest increase in area of the forearm flexors at approximately 72 hours post-injury.

The exact cause of the increase in cross sectional area of the medial gastrocnemius

muscle was not clear from the present data. It is unlikely that increased protein synthesis or connective tissue production were responsible as the area changes were too rapid and too transient. As discussed above, if accumulation of oedema within the muscle was the cause of the increase, a significant increase in T_2 relaxation time would also have been expected but this was not observed. As mentioned above, the small sample size employed in addition to the high variability associated with T_2 measurements may have prevented statistical detection of oedema using T_2 relaxation times.

To summarize, HBO did not have any effect on recovery from any of the variables measured using MRI and MRS techniques, including the ratio of $(P_f/P_i + PCr) * 100$, T_2 relaxation of water protons and cross sectional area of the medial gastrocnemius.

Pain and soreness data:

The patterns of reported pain sensation and unpleasantness over time were very similar in the present study. It is also clear that the magnitude of pain sensation was always greater than its unpleasantness. These phenomena have been reported previously (MacIntyre *et al.*, 1996) and may be due to the fact that because DOMS is commonly experienced, the subjects were less bothered by the discomfort in the knowledge that it would soon subside. Many athletes interpret DOMS as a "positive" form of pain, and hence are less likely to find it unpleasant. Alternatively, it is possible that the affective dimension of DOMS is not prominent compared to other forms of pain.

The reported pain sensation and unpleasantness reached a peak in both groups on

day 2 post-damage, a finding that concurs with other research (MacIntyre *et al.*, 1996). However, the recovery rate from days 2 to 5 appears to be faster in the HBO group compared to the sham group, as reflected by the steeper decline in reported values and also the significantly smaller reported absolute pain sensation and unpleasantness in the HBO group on day 5 post-damage. The HBO group experienced a mean absolute drop in pain sensation from day 2 to day 5 of approximately 92 units as compared to approximately 48 units for the sham group. The corresponding values for unpleasantness were approximately 91 units (HBO group) and 44 units (sham group).

Precisely how HBO might promote this faster recovery from pain and unpleasantness is not clear, particularly in light of the fact that recovery from other measured variables (MRI/MRS and isokinetic data) was not different from days 2 to 5. The subjects were not aware of their treatment conditions and therefore a "Hawthorne effect" was unlikely. The finding may be related to the fact that HBO has been shown to promote the release of the endogenous opiate β-endorphin in healthy adult male athletes (Casti *et al.*, 1993). These investigators determined that HBO (100% oxygen at 2.8 ATA for 60 minutes) had the ability to acutely raise the levels of β-endorphin within the bloodstream even after the tenth consecutive day of treatment. Increased endogenous opiate levels have an analgesic effect and therefore may have reduced the amount of pain and unpleasantness reported by the subjects. It should be noted that Casti *et al.* (1993) determined that HBO had only an acute effect on β-endorphin levels and not a chronic effect (*ie.* a raised basal concentration of β-endorphin). In the present study, pain measures were assessed at a different time of the day from HBO/sham treatments.

Therefore, if analgesia due to raised endogenous opiates was the cause of the reduced pain in the present study, this would imply that the HBO treatments had the ability to raise β-endorphin levels for prolonged periods of time, a conclusion that would not be supported by the findings of Casti *et al.* (1993).

The above discussion concerning the potential analgesic effects of HBO raises an interesting point regarding its use for rehabilitation from sports injuries. In the present study, the subjects given HBO were indicating less pain and unpleasantness on days 3 and 5 than the sham group. However, the functional recovery (isokinetic measures) and biochemical recovery (MRI/MRS measures) at these times did not differ between the groups. A case could be made to suggest that administration of HBO for sports medicine purposes may be counter-productive as an athlete may be tempted to return to sporting activity before the muscle is properly healed. This could increase the likelihood of re-injury and result in a concomitantly longer rehabilitation time.

A further possible explanation of the faster recovery from pain and unpleasantness from days 2 to 5 in the HBO group may be that this was related somehow to the difference in calf muscle size between the groups and not to the HBO treatments themselves. However, this was unlikely given the similarity between groups in reported pain sensation and unpleasantness on days 1 and 2. It is also difficult to justify how muscle size by itself might influence the measurement of or recovery from exercise-induced muscle pain/soreness.

Other than the present research, Staples (1999) and Harrison *et al.* (1999) are the only two studies that have investigated the effect of HBO on recovery from EIMD. Both

studies employed different measures from the present study to gauge the level of pain/soreness that the subjects were experiencing during the days subsequent to muscle damage. Neither were successful in detecting a positive effect of HBO on pain/soreness. Staples (1999) used a lower pressure during HBO (2 ATA) that may not have been sufficient to stimulate the release of endogenous opiates. However, Harrison *et al.* (1999) used the same pressure during HBO as the present study (2.5 ATA) and treatment times were 40 minutes longer (100 minutes per treatment). It is possible that the instruments employed in these studies to measure pain/soreness were not sensitive enough to detect a significant analgesic effect, if one in fact existed.

To summarize, in contrast to previous studies that have investigated the effect of HBO on recovery from EIMD, HBO was found to promote a quicker recovery from pain and unpleasantness from days 2 to 5 post-damage. The exact mechanism responsible for this effect was unclear. As pain is such a subjective experience and thus difficult to measure, it is suggested that further work with larger sample sizes and more sensitive instruments for pain detection should be performed before making any firm conclusions regarding the efficacy of HBO as an analgesic agent.

Conclusions and recommendations for future research:

Numerous mechanisms have been proposed to rationalize the use of HBO in recovery from sports injuries (Staples & Clement, 1996). However, there is very little scientific evidence that supports the use of HBO for sport medicine purposes. This study

was designed to assess whether administration of HBO therapy had the ability to accelerate recovery from muscle damage that had been elicited by a bout of unaccustomed eccentric exercise.

A statistically significant effect of HBO was observed only for three variables in the present study: (i) recovery of peak isometric torque, (ii) recovery from pain sensation and (iii) recovery from pain unpleasantness. The practical significance of a faster recovery from pain and unpleasantness was questionable as the biochemical and functional recovery was not different between the two groups during the same time period. For all other variables measured, recovery was not statistically different between the HBO and sham groups.

Interpretation of the results in this study is limited by the small sample size in each group (n=6). Therefore, the possibility of committing a type II error (accepting the null hypothesis when it is false) was inflated. It is possible that more statistically significant effects of HBO would have been detected with a larger sample size. The small sample size also inflated the possibility that the statistically significant effects noted in this study were not "real" (*ie.* a type I error). Future research of this kind should employ larger sample sizes to minimize the possibility of such errors occurring.

The results of the present study may also have been clearer if the subject characteristics had been more similar between groups. The initial differences (though statistically non-significant) in calf size and strength between the groups cannot be ruled out as contributory cause to some of the results obtained.

On the suggestion of Staples (1999) and Borromeo *et al.* (1997), the present study

employed a higher HBO treatment pressure (2.5 ATA) than previously used in an attempt to maximize the chances of finding a therapeutic effect. The use of even higher pressures in future studies should not be recommended as the chances of complications due to oxygen toxicity would be far greater at pressures approaching and exceeding 3 ATA (Tibbles & Edelsberg, 1996).

It is possible that the time of HBO exposure in the present study (three hours in total) was not sufficient to elicit a significant therapeutic effect. This could be addressed in future studies by increasing either the number of HBO sessions or the time of each session. However, it should be noted that Harrison *et al.* (1999) administered five HBO treatments of 100 minutes each (over eight hours total) and these investigators failed to detect an effect of HBO on recovery from EIMD.

In summary, the results of the present study suggest that HBO is of little benefit for the acceleration of recovery from exercise-induced muscle damage as indicated by the markers evaluated.

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Table 4.1. Subject Characteristics.

Group	Age (yr)	Height (cm)	Body Mass (kg)	Calf raise 1-RM (kg)	Medial gastroc. CSA (cm^2)
HBO [n = 6]	23.8 ± 3.9 (18 - 28)	181.6 ± 3.6 (178.0 - 186.0)	87.3 ± 13.2 (68.0 - 101.0)	302.6 ± 24.0 (272.7 – 329.5)	20.8 ± 4.3 (14.2 - 25.3)
Sham [n = 6]	24.5 ± 2.6 (20 - 27)	179.6 ± 4.8 (173.0 - 185.0)	78.6 ± 8.5 (69.9 - 92.6)	275.0 ± 30.3 (252.3 – 329.5)	15.9 ± 3.5 (12.6 - 19.9)
All [n = 12]	24.2 ± 3.2 (18 - 28)	180.6 ± 4.2 (173.0 - 186.0)	83.0 ± 11.5 (68.0 - 101.0)	288.9 ± 29.7 (252.3 – 329.5)	18.4 ± 4.5 (12.6 - 25.3)

Data are means ± SD, ranges are reported in parentheses.
No significant differences existed between groups.
HBO = Hyperbaric oxygen group.
Sham = sham (placebo) group.
Calf raise 1-RM = the maximum weight that could be lifted for one complete calf raise repetition through a full range of motion (see text).
Medial gastroc. CSA = cross sectional area of the medial gastrocnemius muscle of the right lower leg (measured at the greatest circumference of the calf).

Table 4.2. Values of absolute peak isokinetic torque at 0.52 radians·sec^{-1} (Nm).

Group	Pre	Day 1	Day 2	Day 3	Day 5
HBO [n = 6]	173.0 ± 17.1 [a]	155.8 ± 11.3 [a]	159.0 ± 11.6 [a]	161.2 ± 12.8 [a]	175.0 ± 17.2 [a]
Sham [n = 6]	138.5 ± 15.4	123.2 ± 11.7	124.7 ± 10.8	127.8 ± 17.0	143.2 ± 19.2
All [n = 12]	155.8 ± 23.8	139.5 ± 20.3 [x]	141.8 ± 20.9 [x]	144.5 ± 22.5 [x]	159.1 ± 24.1

Data are means ± SD.
Main effect detected for group and time ($p<0.05$), no interaction.
[a] Significantly different from Sham group ($p<0.05$).
[x] Significantly different from pre and day 5 ($p<0.05$).

Table 4.3. Values of absolute peak isometric torque (Nm).

Group	Pre	Day 1	Day 2	Day 3	Day 5
HBO [n = 6]	182.2 ± 23.1[a]	173.3 ± 18.2[a]	178.3 ± 21.8[a]	182.7 ± 24.1[a]	185.7 ± 16.0[a]
Sham [n = 6]	152.2 ± 28.4	128.8 ± 28.9	139.5 ± 29.2	145.3 ± 27.9	157.0 ± 30.1
All [n = 12]	167.2 ± 29.2	151.1 ± 32.7[x,y]	158.9 ± 31.9[x]	164.0 ± 31.6	171.3 ± 27.4

Data are means ± SD.
Main effect detected for group and time ($p<0.05$), no interaction.
[a] Significantly different from Sham group ($p<0.05$).
[x] Significantly different from pre and day 5 ($p<0.05$).
[y] Significantly different from days 2 and 3 ($p<0.05$).

Table 4.4. Values of absolute isokinetic muscular endurance (Nm).

Group	Pre	Day 1	Day 2	Day 3	Day 5
HBO [n = 6]	1174 ± 125 [a]	1055 ± 193 [a]	1149 ± 153 [a]	1203 ± 207 [a]	1215 ± 235 [a]
Sham [n = 6]	956 ± 150	811 ± 158	877 ± 154	928 ± 208	1003 ± 235
All [n = 12]	1065 ± 174	933 ± 211 [x]	1013 ± 204	1065 ± 245	1109 ± 232

Data are means ± SD.
Main effect detected for group and time ($p<0.05$), no interaction.
[a] Significantly different from Sham group ($p<0.05$).
[x] Significantly different from pre, day 3 and day 5.

Table 4.5. Absolute (P_i/P_i + PCr) * 100 ratio within the medial gastrocnemius muscle.

Group	Pre	Day 1	Day 3	Day 5
HBO [n = 6]	7.7 ± 1.9	12.8 ± 3.5	10.8 ± 3.0	9.6 ± 2.5
Sham [n = 6]	8.2 ± 2.0	13.5 ± 3.0	12.4 ± 2.8	9.6 ± 2.3
All [n = 12]	7.9 ± 1.9	13.1 ± 3.1 [x]	11.6 ± 2.9 [x]	9.6 ± 2.3 [y]

Data are means ± SD.
Main effect detected for time ($p<0.05$), no interaction.
[x] Significantly different from pre and day 5 ($p<0.05$).
[y] Significantly different from pre ($p<0.05$).

Table 4.6. T_2 relaxation times (in ms) determined using mono-exponential and multi-exponential fitting techniques.

Subject	Group	T_2 technique	Pre	Day 1	Day 3	Day 5
1	HBO	Mono[1]	35.3	38.5	34.5	33.3
		NNLS[2]	**27.2 (86.2%)**	**39.1 (100%)**	**33.4 (100%)**	**27.6 (92.0%)**
			56.5 (13.8%)			**58.4 (8.0%)**
2	HBO	Mono	35.6	43.7	119.7	50.8
		NNLS	**33.4 (100%)**	**36.2 (100%)**	**118.8 (100%)***	**32.3 (77.7%)**
						89.5 (22.3%)
3	HBO	Mono	40.4	55.6	43.4	39.8
		NNLS	**29.3 (92.3%)**	**31.8 (92.9%)**	**27.9 (100%)**	**28.1 (100%)**
			101.6 (7.7%)	**138.6 (7.1%)**		
4	HBO	Mono	33.3	37.6	39.1	40.7
		NNLS	**30.5 (100%)**	**29.4 (98.3%)**	**29.8 (100%)**	**30.0 (100%)**
				71.4 (1.7%)		
5	HBO	Mono	35.2	36.8	35.7	36.2
		NNLS	**28.1 (100%)**	**32.1 (100%)**	**27.9 (99.0%)**	**28.1 (99.5%)**
					123.3 (1.0%)	**235.3 (0.5%)**
6	HBO	Mono	43.3	40.1	44.5	41.2
		NNLS	**28.2 (89.6%)**	**32.3 (99.2%)**	**31.3 (100%)**	**30.5 (99%)**
7	Sham	Mono	35.6	43.7	119.7	50.8
		NNLS	**31.4 (100%)**	**35.9 (100%)**	**24.9 (58.6%)**	**31.1 (92.4%)**
					40.0 (41.1%)	**76.3 (7.6%)**
8	Sham	Mono	32.3	34.9	35.2	42.1
		NNLS	**40.5 (100%)**	**33.4 (100%)**	**34.8 (100%)**	**39.9 (100%)**
9	Sham	Mono	32.6	40.0	63.9	54.2
		NNLS	**30.6 (100%)**	**34.1 (100%)**	**36.4 (93.4%)**	**35.0 (90.8%)**
					224.1 (6.6%)	**112.8 (9.2%)**
10	Sham	Mono	40.2	41.2	38.6	42.2
		NNLS	**34.7 (100%)**	**30.7 (90.4%)**	**28.5 (97.9%)**	**24.9 (62.5%)**
				67.4 (9.6%)	**109.8 (2.1%)**	**42.9 (37.5%)**
11	Sham	Mono	40.1	35.8	40.4	35.3
		NNLS	**28.7 (98.9%)**	**27.9 (99.5%)**	**26.4 (97.0%)**	**27.8 (100%)**
			191.6 (1.1%)		**109.7 (3.0%)**	
12	Sham	Mono	66.9	59.8	41.7	46.1
		NNLS	**33.3 (84.9%)**	**38.5 (90.5%)**	**32.4 (96.8%)**	**32.9 (93.6%)**
			128.6 (15.1%)	**138.5 (9.5%)**	**104.8 (3.2%)**	**96.3 (6.4%)**
Mean HBO group (± SD)		Mono	37.2 ± 3.8	42.1 ± 7.1	39.4 ± 4.5	40.3 ± 6.0
		NNLS[3]	**29.5 ± 2.2**	**33.5 ± 3.5**	**30.1 ± 2.4**	**29.4 ± 1.8**
Mean Sham group (± SD)		Mono	41.0 ± 13.2	41.3 ± 9.4	42.3 ± 11.0	42.9 ± 6.7
		NNLS[3]	**33.2 ± 4.1**	**33.4 ± 3.7**	**30.6 ± 4.7**	**31.9 ± 5.3**
Mean both groups (± SD)		Mono	39.1 ± 9.5	41.7 ± 7.9	41.0 ± 8.4	41.6 ± 6.2
		NNLS[3]	**31.3 ± 3.7**	**33.5 ± 3.5**	**30.3 ± 3.6**	**30.7 ± 4.0**

Data in **bold** represents T_2 values measured using the NNLS technique. The values in parentheses represent the proportion of the T_2 decay contributed by that component.

[1] T_2 determined using a mono-exponential fitting technique.

[2] T_2 determined using a non negative least squares (NNLS) algorithm for multi-exponential fitting.

[3] In the cases where there are two NNLS T_2 components only the shortest one (between 20-40 ms) was used to calculate the mean value. * This data point was disregarded as an outlier.

No significant differences in T_2 (mono) or T_2 (NNLS) were detected over time or between groups.

Table 4.7. Absolute cross sectional area of the medial gastrocnemius muscle (cm^2)

Group	Pre	Day 1	Day 3	Day 5
HBO [n = 6]	20.8 ± 4.3 [a]	22.0 ± 4.4 [a]	21.6 ± 4.0 [a]	20.9 ± 4.3 [a]
Sham [n = 6]	15.9 ± 3.5	16.7 ± 3.6	16.2 ± 3.1	16.2 ± 3.3
All [n = 12]	18.4 ± 4.5	19.4 ± 4.7 [x]	18.9 ± 4.5	18.5 ± 4.4

Data are means ± SD.
Main effect detected for time and group ($p<0.05$), no interaction.
[a] Significantly different from Sham group ($p<0.05$).
[x] Significantly different from pre and day 5 ($p<0.05$).

Table 4.8. Absolute cross sectional area of the tibial bone marrow (cm^2)

Group	Pre	Day 1	Day 3	Day 5
HBO [n = 6]	2.5 ± 0.9	2.5 ± 0.9	2.4 ± 0.9	2.4 ± 0.9
Sham [n = 6]	2.5 ± 0.9	2.5 ± 0.8	2.5 ± 0.9	2.5 ± 0.8
All [n = 12]	2.5 ± 0.9	2.5 ± 0.8	2.5 ± 0.8	2.5 ± 0.8

Data are means ± SD.
No significant differences were observed.

Table 4.9. Absolute reported pain sensation as measured by the Descriptor Differential Scale (arbitrary units – see text).

Group	Day 1	Day 2	Day 3	Day 5
HBO [n = 6]	136.8 ± 37.9	150.5 ± 57.2	111.5 ± 46.6 [h]	58.2 ± 38.3 [f,g]
Sham [n = 6]	109.6 ± 38.5	132.2 ± 33.5	124.6 ± 33.8	83.8 ± 13.9 [g]
All [n = 12]	124.5 ± 38.9	140.5 ± 44.9 [x]	115.1 ± 38.9	70.3 ± 30.1 [x]

Data are means ± SD.
Interaction detected ($p<0.05$).
[f] Significantly different from Sham day 5 ($p<0.05$).
[g] Significantly different from all other intragroup days ($p<0.05$).
[h] Significantly different from intragroup day 2 ($p<0.05$).
[x] Significantly different from all other days ($p<0.05$).

Table 4.10. Absolute reported unpleasantness as measured by the Descriptor Differential Scale (arbitrary units – see text).

Group	Day 1	Day 2	Day 3	Day 5
HBO [n = 6]	96.3 ± 29.4	127.0 ± 59.8	81.7 ± 39.0 [l]	36.2 ± 28.8 [i,j]
Sham [n = 6]	83.3 ± 34.8	108.0 ± 37.2	98.2 ± 41.7	63.8 ± 28.1 [k]
All [n = 12]	89.8 ± 31.4	117.5 ± 48.5 [x]	89.9 ± 39.5	50.0 ± 30.7 [x]

Data are means ± SD.
Interaction detected ($p<0.05$).
[i] Significantly different from Sham day 5 ($p<0.05$).
[j] Significantly different from all other intragroup days ($p<0.05$).
[k] Significantly different from intragroup day 2 ($p<0.05$).
[l] Significantly different from intragroup day 2 ($p<0.05$).
[x] Significantly different from all other days ($p<0.05$).

	←Baseline testing→		Muscle damage	←Post damage treatment/testing period→			
Day	Mon (Pre 1)	Tues (Pre 2)	Wed (Day 0)	Thurs (Day 1)	Fri (Day 2)	Sat (Day 3)	Mon (Day 5)
Damage protocol			✓				
MRI/ MRS		✓		✓		✓	✓
DDS				✓	✓	✓	✓
Isokinetic testing	✓	✓		✓	✓	✓	✓
HBO/ Sham			✓ (3-4h after damage)	✓ (24h after 1st HBO)	✓ (48h after 2nd HBO)		

MRI/MRS - Magnetic resonance imaging and spectroscopy testing.
DDS - Descriptor Differential Scale.
HBO/Sham - HBO or sham treatment (dependent on group assignment).

Figure 4.1. Summary of the experimental design.

Figure 4.2. Standing calf raise machine on which the exercise protocol was performed.

Figure 4.3. A monoplace hyperbaric chamber within the Misericordia Hospital, Edmonton.

Figure 4.4. Position assumed for isokinetic testing of the calf muscle.

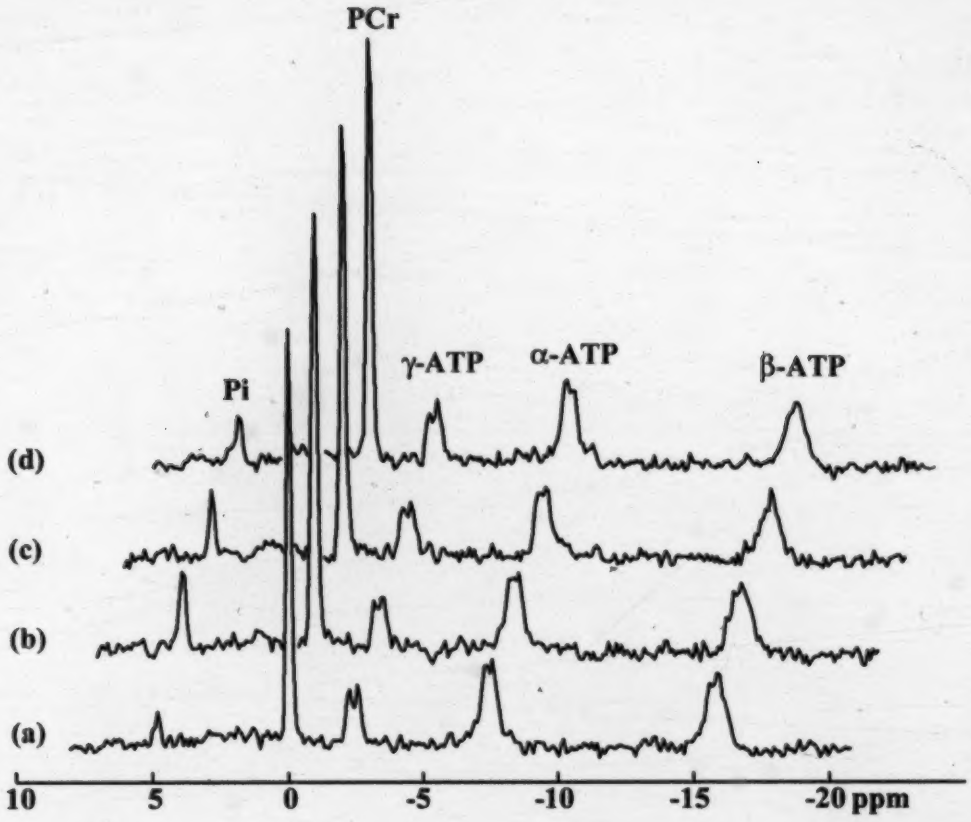

Figure 4.5. Stacked ^{31}P spectra for subject 7 demonstrating the changes that occur in the size of the P_i peak after exercise-induced muscle damage.
Spectra (a), (b), (c) and (d) correspond to those obtained at pre, day 1, day 3 and day 5 respectively.
Note: Images (b), (c) and (d) are offset to the right for the purpose of illustration only.

Figure 4.6. Location of the area for investigation within the gastrocnemius muscle (subject 7).
The rectangle represents the STEAM selected volume within the medial gastrocnemius that was used for measurement of T_2 relaxation time.
The parallel lines represent the ISIS 1D slice that was used for ^{31}P measurement within the medial gastrocnemius.

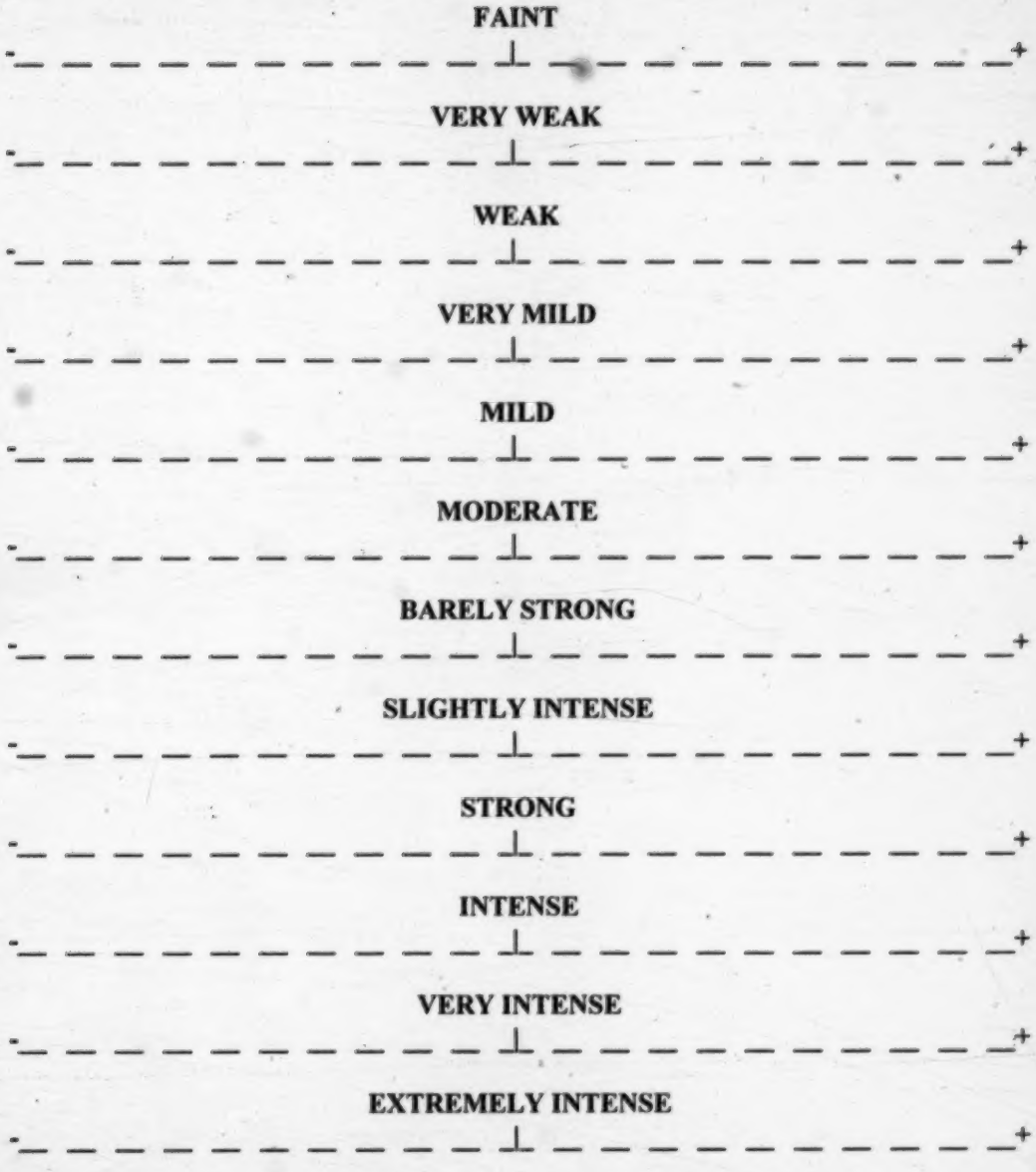

Figure 4.7. The sensory intensity form of the Descriptor Differential Scale (in order of increasing magnitude).

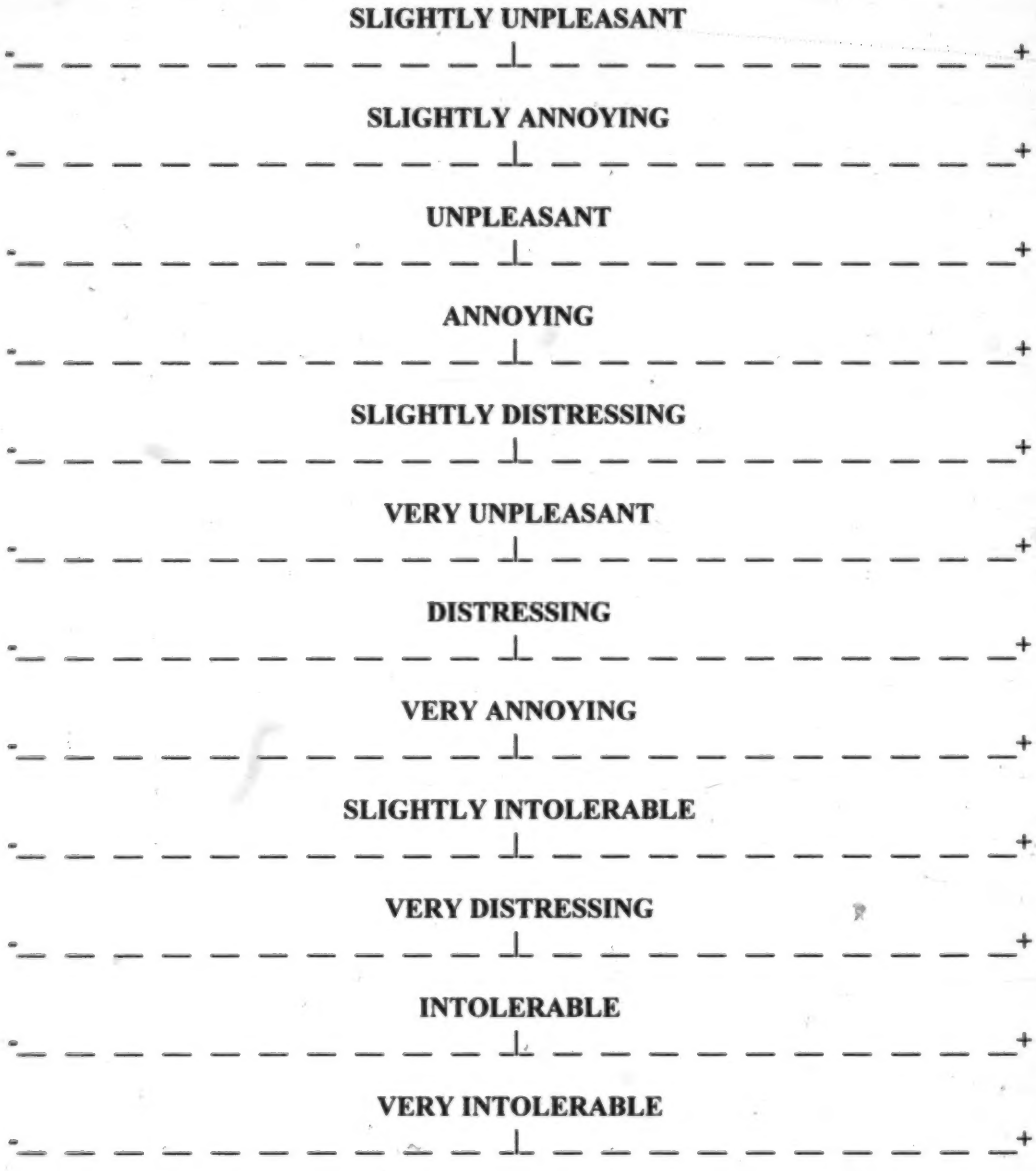

Figure 4.8. The unpleasantness form of the Descriptor Differential Scale (in order of increasing magnitude).

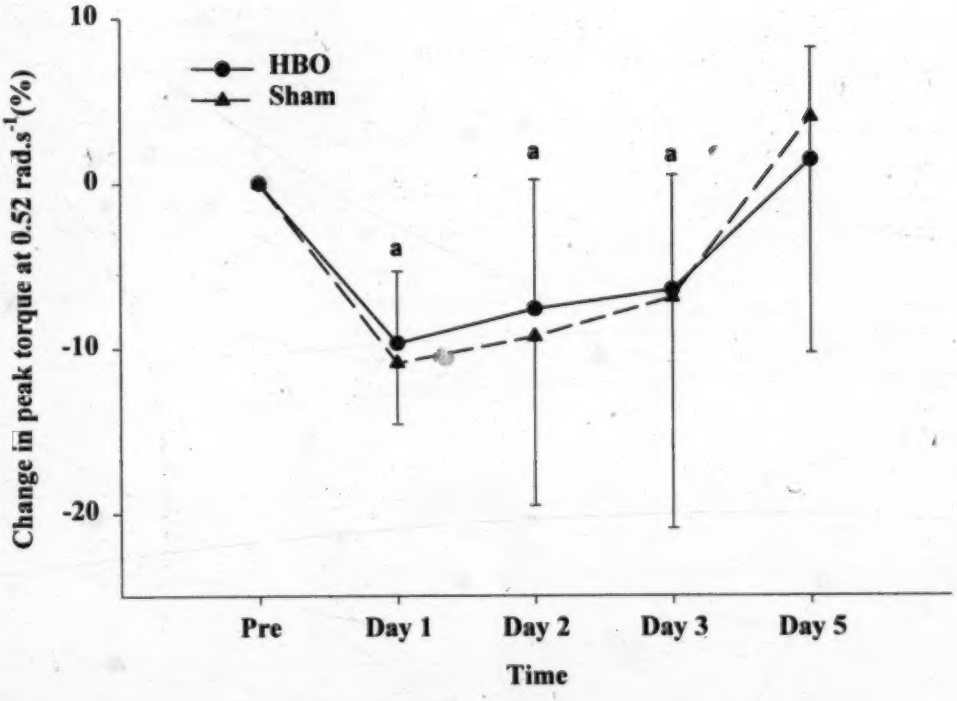

Figure 4.9. Percentage change over time in peak isokinetic torque at 0.52 rad.s^{-1}

Main effect for time ($p<0.05$).
a - both groups significantly different from pre and day 5 ($p<0.05$).
Bars = SD.

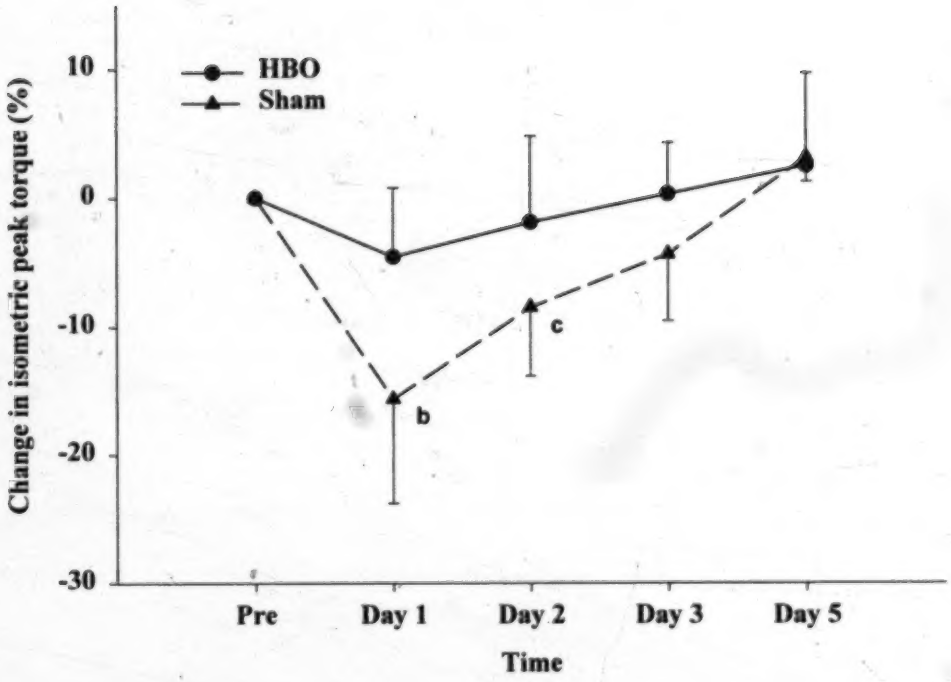

Figure 4.10. Percentage change over time in isometric peak torque.

Significant interaction detected ($p<0.05$).
b - significantly different from all others ($p<0.05$).
c - significantly different from all others except day 1 & 2 (HBO) and day 3 (Sham) ($p<0.05$).
Bars = SD.

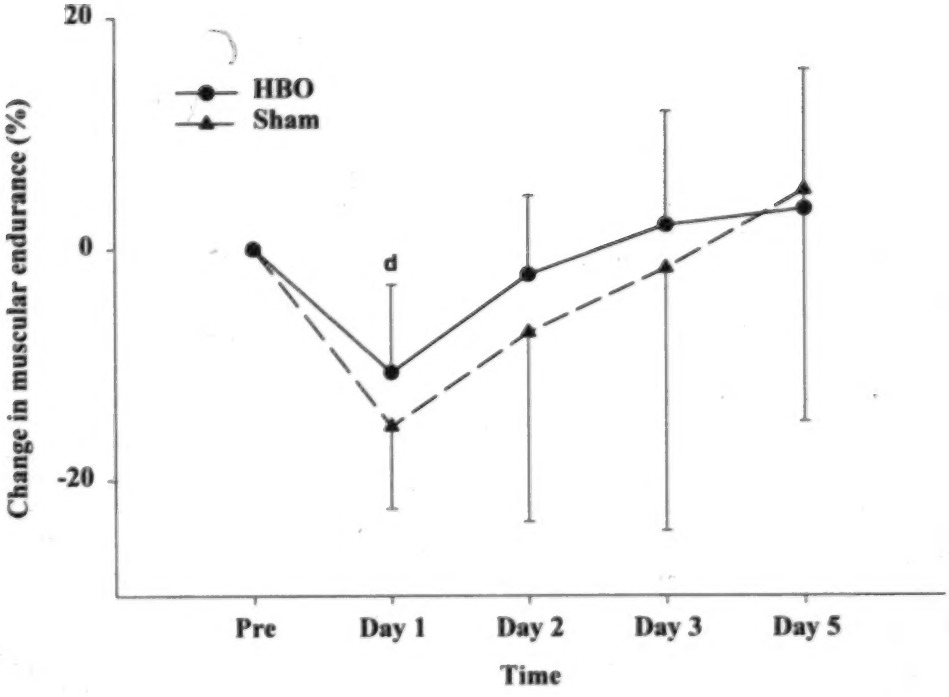

Figure 4.11. Percentage change over time in isokinetic muscular endurance.

Main effect for time ($p<0.05$).
d - both groups significantly different from all other days ($p<0.05$).
Bars = SD.

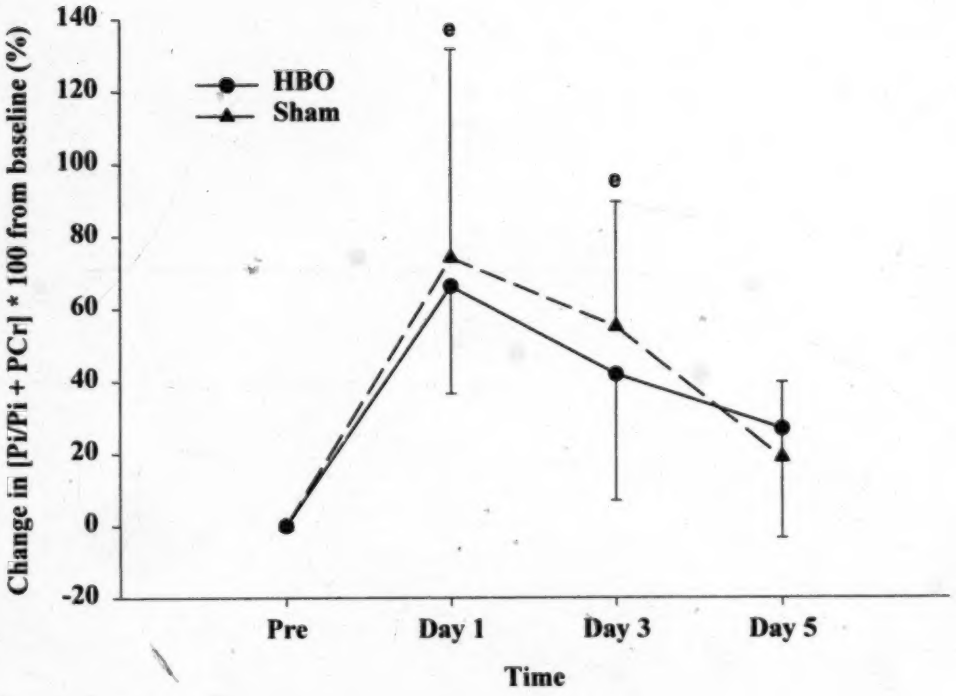

Figure 4.12. Percentage change in [Pi/Pi + PCr] * 100 over time.

Main effect for time ($p<0.05$).
e - both groups significantly different from pre and day 5.
Bars = SD.

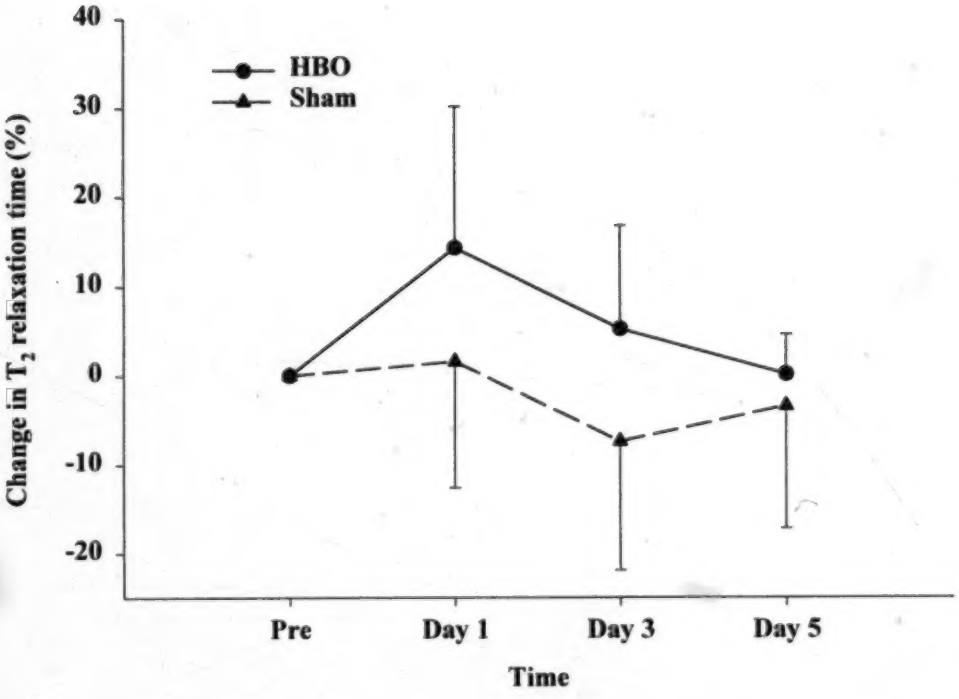

Figure 4.13. Percentage change in T_2 relaxation time determined using multiexponential (NNLS) fitting.

No significant differences were detected.
Bars = SD.

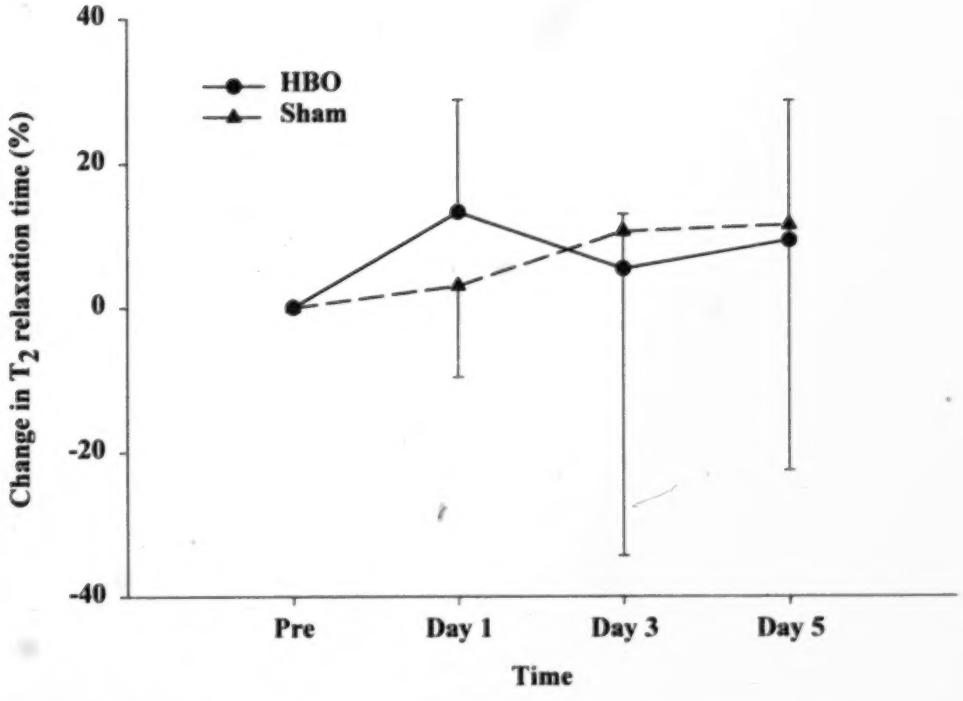

Figure 4.14. Percentage change over time in T_2 relaxation time determined using monoexponential fitting.

No significant differences were detected.
Bars = SD.

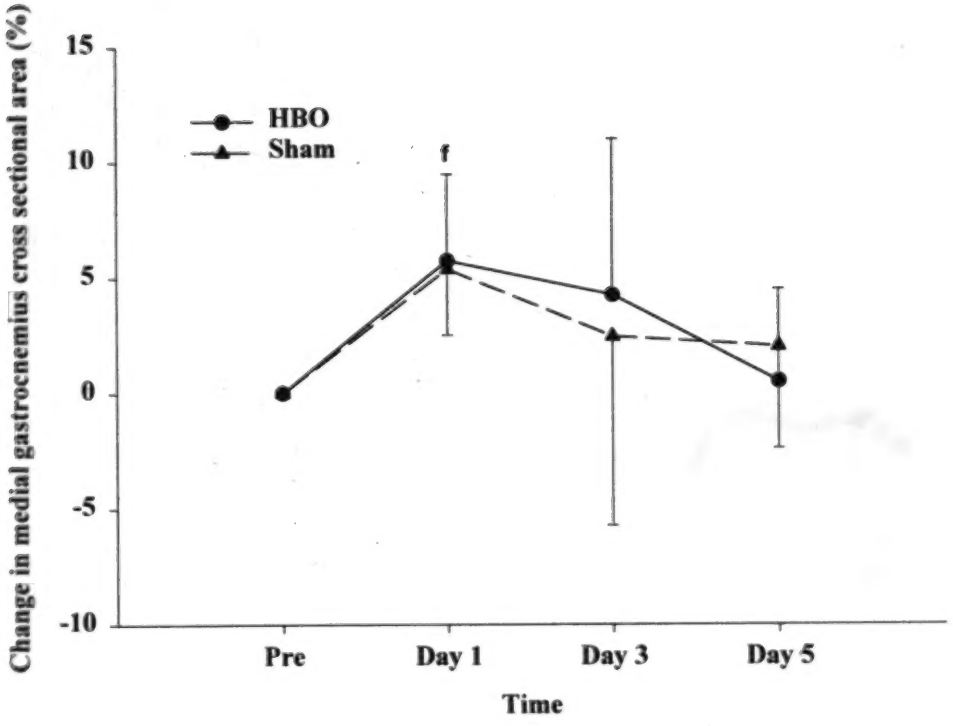

Figure 4.15. Percentage change in medial gastrocnemius cross sectional area over time.

Main effect for time ($p<0.05$).
f - both groups significantly different from pre and day 5.
Bars = SD.

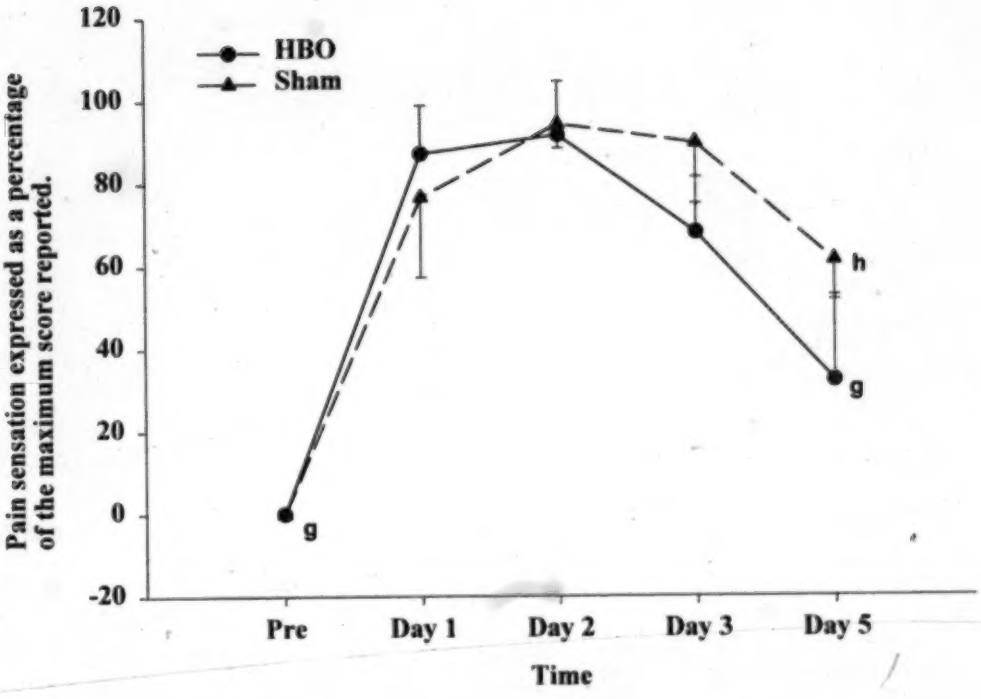

Figure 4.16. Reported pain sensation expressed as a percentage of each subject's maximum reported score.

Significant interaction detected ($p<0.05$).
g - significantly different from all others ($p<0.05$).
h - significantly different from all others except day 3 (HBO) and day 1 (Sham)
Bars = SD.

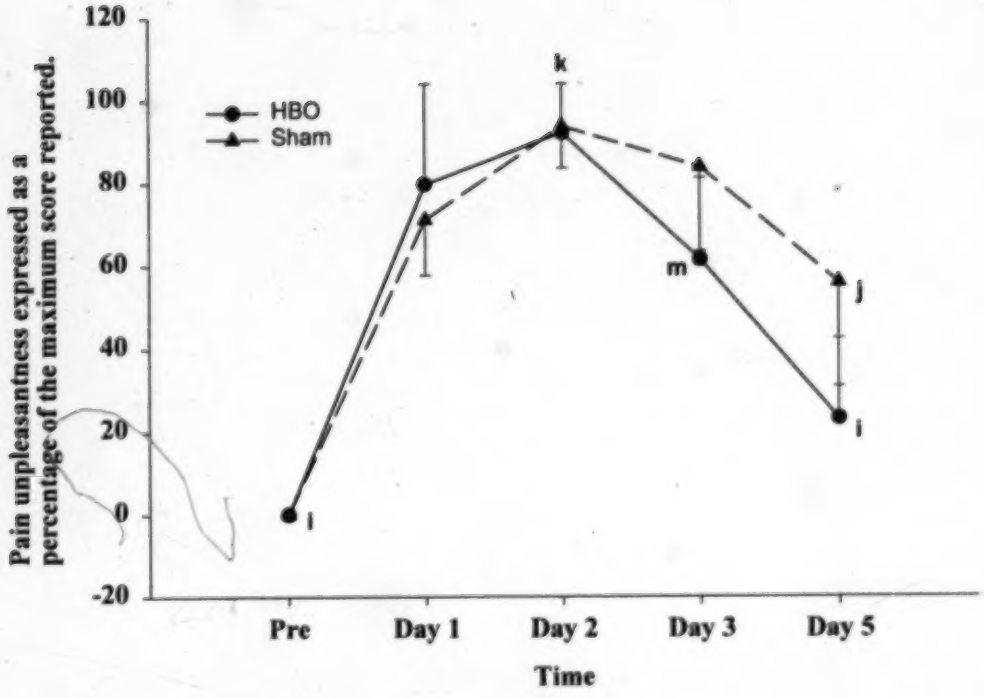

Figure 4.17. Reported pain unpleasantness expressed as a percentage of each subject's maximum reported score.

Significant interaction detected ($p<0.05$).
i - significantly different from all others ($p<0.05$).
j - significantly different from day 2 (HBO & Sham) and day 5 (HBO).
k - both points significantly different from day 3 (HBO) and day 5 (HBO & Sham).
m - significantly different from day 2 (HBO & Sham) and day 5 (HBO).
Bars = SD.

CHAPTER FIVE

General Discussion and Conclusions.

Hyperbaric oxygen (HBO) is an established treatment for a number of clinical conditions including decompression sickness, air embolism and severe carbon monoxide poisoning (Grim *et al.*, 1990; Tibbles & Edelsberg, 1996). Most of the beneficial effects of the therapy are due to hyperoxia, or increased partial pressures of oxygen within the tissues of the body. There are numerous biochemical and physiological consequences of increased levels of oxygen within the body and, approximately a decade ago, some proposed that hyperbaric hyperoxia might have a role to play as an adjuvant therapy in rehabilitation and sports medicine (Jain, 1990).

Hardly surprisingly, given the propensity of elite athletes and coaches to attempt to gain an "edge" on the competition, it did not take long for the sports medicine community to take an interest in HBO. In the early 1990's, reports began to emerge in the popular press that professional athletes were undergoing hyperbaric therapy for either (or both) of two purposes: first, as an ergogenic aid to improve subsequent performance (Potera, 1995) or, second, to accelerate recovery from sports injuries (James *et al.*, 1993; Staples & Clement, 1996). Unfortunately, most of these reports were anecdotal in nature and there was little rigorous scientific evidence to support the use of HBO for these purposes.

HBO therapy is not only expensive, it is also potentially hazardous, especially when administered by unqualified personnel. Aside from the obvious risk of fire and explosion, its major complications include barotrauma as a result of the mechanical effect of increasing pressure and also the potentially serious toxic effects of increased concentrations of oxygen (Grim *et al.*, 1990; Tibbles & Edelsberg, 1996). The latter complication in particular is one of which many are unaware. It is not unusual to see

images of athletes in various sports breathing supplemental oxygen in an effort to speed recovery. Somewhat understandably, many have therefore assumed that "if some is good, more is better". In reality, the exact opposite is true.

Very little research has been able to demonstrate that HBO has a beneficial effect in the area of sports medicine, either for performance enhancement or for recovery from injury. Given the potentially serious complications and cost of HBO, it was clear that additional work needed to be done to elucidate the potential role of this therapy in sports medicine.

The purpose of the research detailed in this thesis was to clarify two potential roles of HBO in the sports medicine setting. Study 1, detailed in Chapter III, was designed to ascertain whether exposure to HBO prior to an incremental exercise test had the ability to improve measured physiological indices during that performance. Study 2, detailed in Chapter IV, was designed to ascertain whether administration of HBO had the ability to accelerate recovery from exercise-induced muscle damage (EIMD) elicited by a bout of strenuous unaccustomed exercise.

No evidence of an ergogenic effect of HBO (100% oxygen at 2.0 ATA for 60 minutes) prior to performance was found in study 1. The major physiological measures taken (VO_2max, ventilation threshold and lactate threshold) demonstrated no change during the test that was preceded by HBO when compared to the baseline tests with no prior HBO. The only variable that did demonstrate a difference after HBO was muscle oxygenation as measured by near-infrared spectroscopy, but a statistically significant difference was detected only at one power output during the exercise test (235W). The

cause of this difference was unclear but it was unlikely, in view of the fact that all other physiological measures were unchanged, that it had significant ergogenic implications.

Critics of the above study might argue that no ergogenic effect of prior HBO was noted for any (or all) of several reasons. First, the pressure at which HBO was administered (2.0 ATA) may have been insufficient to elicit an effect. Second, the length of the HBO treatment (60 minutes) may have been insufficient. Lastly, the time period between cessation of HBO and performance of exercise (22.5 ± 5.6 minutes) may have been too long for a potential ergogenic effect to have persisted. In response to the first two concerns, the results of McGavock *et al.* (1999) can be cited. These investigators examined the effects of HBO treatment (90 minutes at 2.5 ATA) on subsequent aerobic performance (running economy, VO_2max and run time to exhaustion) in a trained population. Despite the longer treatment time and higher pressure used, no significant effect of HBO was seen for any of the variables measured. The use of even higher pressures than 2.5 ATA for future studies cannot be recommended due to the increased risk of oxygen toxicity. In response to the last concern of an excessive period of time between HBO and performance, attention should be paid to the calculation contained in Appendix A. This demonstrates that the "extra" oxygen contained within the body on cessation of HBO would be consumed by the resting body within seconds, not minutes. Therefore, it is highly unlikely that HBO can have any ergogenic effect by oxygen retention.

Study 2 utilized EIMD as a model of muscle injury and investigated the effect of three HBO treatments (each for 60 minutes at 2.5 ATA) on recovery from this damage. Measures that were used to monitor recovery included muscle function via isokinetic

dynamometry, muscle cross sectional area using magnetic resonance imaging (MRI), inorganic phosphate and T_2 relaxation time using ^{31}P and ^{1}H magnetic resonance spectroscopy (MRS) respectively and measures of pain sensation and unpleasantness. A control group was included in this study that received a "sham" treatment instead of HBO. The results of the study indicated that there was little evidence of a difference in recovery rate between the groups. Faster recovery was observed for three variables only: isometric peak torque, pain sensation and pain unpleasantness. The mechanism underlying the faster recovery in isometric peak torque was unclear, particularly as there were no differences in recovery between the groups for the other isokinetic measures taken. The reason for the faster recovery from pain sensation and unpleasantness was also not clear. However, the practical importance of this finding is questionable in view of the fact that other measures of functional and biochemical recovery of the muscle were not different. In summary, study 2 failed to provide compelling evidence that HBO was a useful method of treatment of EIMD.

It could be argued that the reason for the lack of significant results in study 2 was insufficient total HBO exposure time (3 treatments x 60 minutes = 180 minutes). However, Harrison *et al.* (1999), using a similar experimental protocol to the present study, determined that 500 minutes of total HBO exposure time over the five days post-muscle damage was also insufficient to elicit any evidence of accelerated recovery from EIMD.

The lack of compelling evidence in the study for the efficacy of HBO in recovery from EIMD does not preclude the possibility that it may be of benefit for other forms of soft tissue "sport" injuries such as strains, sprains and tendonitis. Very little work has

been performed on humans to investigate the efficacy of HBO for recovery from these injuries. Further work needs to be done, using larger sample sizes and control conditions, to investigate whether there may be a role for HBO in these instances.

To summarize, the research performed in this thesis failed to demonstrate that HBO has a significant role to play in sports medicine either as an ergogenic aid prior to performance or as a therapeutic modality for acceleration of recovery from EIMD. Therefore, particularly in view of the costs and potential complications of HBO, its use within sports medicine is unjustified and should be discouraged. Athletes would be far better advised to pursue other more traditional and proven methods of rehabilitation from soft tissue injuries sustained as a result of sport performance.

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APPENDICES

Chapter Three

Appendix A: Calculation of time required for excess dissolved oxygen in the bloodstream to be utilized on emergence from HBO at 2 ATA.

Appendix B: Informed consent form and participant information sheet for study 1.

Chapter Four

Appendix C: Notice used for recruitment of subjects for study 2.

Appendix D: Questionnaire concerning calf training status for study 2.

Appendix E: Questionnaire for detection of contra-indications to HBO.

Appendix F: Questionnaire for detection of contra-indications to MRI.

Appendix G: Participant information letter for study 2.

Appendix H: Informed consent form for study 2.

Appendix I: Literature review of the causes and consequences of exercise-induced muscle damage

Appendix A:

[This calculation was performed at the request of the reviewers of *Undersea and Hyperbaric Medicine* and was submitted with the revised manuscript in a covering letter to the journal].

Calculation of the time required for oxygen levels to return to "normal" after HBO:

- Partial pressure of O_2 in chamber at 2 ATA at sea level = 760 x 2 =1520mmHg.
- Partial pressure of water vapour at body temp. = 47mmHg $\therefore$ $P_I O_2$ = 1520 - 47 = 1473mmHg
- $P_AO_2 = P_IO_2 - P_aCO_2$ (when breathing 100% O_2)
  $= 1473 - 38 = 1435$mmHg
- Assume P_aO_2/P_AO_2 ratio of 0.88 for 25 year old males (R. Jones [co-author], personal communication)
- Therefore, estimated $P_aO_2 = 1435 \times 0.88 = 1263$mmHg
- The amount of oxygen that will be physically dissolved in arterial plasma at this P_aO_2 = 1263 x 0.0031 = 3.9 ml/100ml arterial plasma.
- According to Rowell (1993)*, no more than 25% of the total blood volume is found within the arterial system at any one time. Therefore, if one were to assume a total blood volume in a male individual of 5 litres, this would constitute an arterial blood volume of 5 x 0.25 = 1.25 litres.
- Therefore, total volume of oxygen dissolved in arterial blood = 3.9 x 1.25 x 10 = 49ml dissolved O_2.
- Assume resting metabolism of 1 MET (equivalent to 3.5 ml $O_2 \cdot kg^{-1} \cdot min^{-1}$). This corresponds to an absolute resting oxygen consumption in an "average" male of 70kg of 3.5 x 70 = 245 $ml \cdot min^{-1}$
- Therefore, one can expect the dissolved oxygen within arterial blood to be consumed in approximately 49/245 = 0.2 minutes ≡ 12 seconds.

* Rowell L.B. (1993). Human Cardiovascular Control. New York: Oxford University Press. p.3-36.

This calculation only takes into account oxygen dissolved within plasma and does not include an estimation of the extra oxygen that would be bound to myoglobin and also that dissolved within intra- and extracellular fluid. It is only an approximate value and should be viewed with some caution. However, it serves to demonstrate that the period of "oxygen retention" after HBO treatment is likely extremely brief and impractical as a means of enhancing subsequent performance.

Appendix B:

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THE EFFECT OF ACUTE HYPERBARIC OXYGENATION ON ANAEROBIC THRESHOLD, VO_2 MAX AND PULMONARY FUNCTION.

Investigators:

(1) Tony Webster, M.Sc.	(403) 436-2184
(2) Dan Syrotuik, Ph.D.	(403) 492-1018
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(4) Brian Fisher, Ph.D.	(403) 492-8273
(5) Malcolm Young, M.D.	(403) 930-5717
(6) Dick Jones, Ph.D.	(403) 492-6475.

Investigators 1 to 4 are affiliated to the Faculty of Physical Education and Recreation at the University of Alberta. Investigators 2 to 4 are staff members of the Faculty and the first is a provisional Ph.D. student in the Faculty. Investigator 5 is an emergency care physician at the Misericordia Hospital, Edmonton. Investigator 6 is affiliated to the Faculty of Medicine at the University of Alberta. The project is intended to be a pilot study as part of the first investigator's Ph.D program.

INFORMED CONSENT FOR PARTICIPATION IN THE ABOVE STUDY

I,_____(please print your name) agree to participate in a research project conducted by the above named investigators studying the effect of an acute bout of hyperbaric oxygenation on anaerobic threshold, VO_2max and pulmonary function. I agree to participate in all procedures to the best of my ability. I understand that I may withdraw from the study at any time, or discontinue any test procedure at my own free will. I also understand that the staff conducting the tests/procedures will discontinue any procedures if any indications of abnormal responses become apparent. I understand that prior to performing any test listed below or at any stage during the study I will have the opportunity to question and discuss the exact procedures to be followed.

Purpose of the study:

The primary purpose of this study will be to determine the effects of an acute bout of hyperbaric oxygenation on subsequent aerobic endurance performance in a normobaric environment (*ie.* "normal " conditions). Measurements made to indicate aerobic endurance performance will include an anaerobic threshold and VO_2max test. The study will also investigate whether a bout of hyperbaric oxygenation has the ability to alter pulmonary (lung) function.

Research procedures:

(1) VO_2max/anaerobic threshold tests - all potential subjects will be required to perform ***three*** identical cycle exercise tests designed to assess VO_2max and anaerobic threshold (AT). The tests will be performed on three separate days. Two will occur before exposure to hyperbaric oxygen (HBO) and one immediately after the HBO exposure (two tests prior to HBO are required for the purpose of assessing the variability/stability of VO_2max and AT). Subjects will be required to meet a certain aerobic fitness level (*ie.* 55ml.kg.$^{-1}$min^{-1}) if they are to be included in the study. The tests will take the form of a continuous exercise test of increasing intensity to exhaustion on a cycle ergometer. Metabolic responses and heart rate will be monitored during the test with the aid of a Horizon metabolic measurement cart and Polar heart rate monitor. Blood will be drawn at regular periods during these tests (to measure blood lactate) by means of an indwelling venous catheter inserted into an antecubital (*ie.* forearm) vein.

During each exercise test, levels of oxygen in arterial blood will be monitored by means of a transcutaneous oxygen sensor. This is a simple non-invasive procedure whereby a small heated sensor is placed on the skin of the forearm.

(2) Pulmonary function tests - subjects will perform ***three*** identical pulmonary (lung) function tests designed to measure vital capacity, diffusing capacity and functional residual capacity. These tests will be performed on the same days as, and immediately prior to, the exercise testing sessions. Hence, two of these tests will be performed before HBO and one immediately after HBO.

(3) Hyperbaric oxygenation - subjects will undergo an acute bout of HBO in a hyperbaric chamber at the Misericordia Hospital, Edmonton. The procedure will be performed by highly trained medical technicians. Compression to 2 atmospheres absolute (2 ATA) will take place over a period of about 10 minutes (2 ATA is the pressure equivalent to submerging 10 meters under water). On completion of compression, subjects will be given 100% oxygen to breathe for a period of 60 minutes. Subjects will be lying peacefully in the chamber for the duration of the treatment. After 60 minutes subjects will be decompressed to 1 ATA (normal atmospheric pressure) over a period of about 10 minutes.

(4) As outlined above, immediately after the HBO bout, subjects will undergo a third pulmonary function and anaerobic threshold/VO_2max test to determine if any changes have occurred in these variables. The exact time after HBO that these tests will be administered will be verified at a later time when the logistics of the procedures have been determined but it will be less than an hour after HBO.

It is anticipated that the whole protocol will require no more than 6 hours with the subjects being required for at least one hour on two separate occasions and 3 hours on a third occasion.

Risks to subjects:

The exercise tests will require maximal physical and mental effort. However, the effort required will not be greater than that experienced during some sport performances. The tests represent little risk to healthy, active individuals involved in sport.

The venous catheter inserted for the purpose of sampling blood lactate may represent some discomfort to the subjects but the procedure will be performed safely by trained medical/nursing personnel and so should represent no risk to the subjects. Some minor bruising may be expected at the puncture site after the catheter is removed.

The hyperbaric oxygenation will be performed in the Misericordia Hospital, Edmonton by highly trained medical technicians. There are a number of relative contraindications for hyperbaric oxygenation and so individuals must be screened for these before they may undergo the procedure. These contraindications include upper respiratory tract infections, seizure disorders (such as epilepsy), emphysema, severe asthma, pulmonary lesions, a history of thoracic or ear surgery, uncontrolled high fever, malignant disease and optic neuritis. Subjects are required to complete a questionnaire to confirm that they are not suffering from any of these conditions and they must also undergo a medical examination. The presence of any of these contraindications will result in the subject being disallowed to continue with the study (except in the case of a subject developing an infection, in which case postponement of HBO until the symptoms have resided would be appropriate).

The most common complications associated with HBO involve cavity trauma due to changes in external pressure. For example, pain may arise as a result of blocked sinuses or from an inability to equalize middle ear pressure with that of outside air during compression. Subjects can remedy this by Frenzel's manoeuver (pinching the nose, closing the mouth and pushing the tongue against the soft palate to force air through the Eustachian tubes into the middle ears). Frenzel's manoeuver is commonly known as "popping" one's ears. Prior studies have indicated that the chances of a healthy subject experiencing problems with cavity trauma are around 1 in 300.

Oxygen at high concentrations is toxic to the body if the body is exposed for prolonged periods. Central nervous system oxygen toxicity is a problem mainly associated with prolonged HBO treatment sessions at relatively high pressures (*ie.* greater than 3 ATA). At the pressure being used in the present study (2 ATA) it has been estimated that the chance of experiencing any form of oxygen toxicity is less that 1 in 5,000. In the unlikely event of a subject experiencing oxygen toxicity, the fraction of inspired oxygen

will be decreased to normal levels (*ie*. 21% of inspired air). This procedure is sufficient to cause symptoms to resolve and they will generally not recur if 100% oxygen is restarted. Even if a subject does experience oxygen toxicity, there are no lasting side effects.

In summary, no serious complications are associated with moderate pressure and duration HBO treatments such as that being used in the present study.

Consent:

I acknowledge that I have read this form and I understand the test procedures to be performed and the inherent risks and benefits involved from participation in this project. I consent to participate understanding that I may withdraw at any time without prejudice. I acknowledge my obligation to inform the testing personnel of any pain, discomfort or abnormal sensations that I might experience during or after the tests. I may expect a copy of this consent form and a report of my personal results after the study is complete and understand that the data collected will be used for a research project and will remain in the possession of the investigator to ensure confidentiality and anonymity. I also understand that I may make enquiries at any time during the project concerning any procedures that I do not completely understand.

I consent to participate in this research project.

Name:_____ Signature:_____ Date:_____

Address:_____ Postal code:_____

Phone: (Home)_____(Work)_____ Age:_____

Witness:_____ Investigator:_____

Appendix C:

SUBJECTS NEEDED!

FOR PARTICIPATION IN A RESEARCH STUDY ENTITLED "EFFECTS OF HYPERBARIC OXYGEN ON RECOVERY FROM EXERCISE-INDUCED MUSCLE DAMAGE"

Researchers in the Faculty of Physical Education and Recreation at the University of Alberta will be examining the effects of treatment within hyperbaric chambers on recovery from muscle damage and muscle soreness obtained as a result of strenuous muscular exercise.

- **If you are male between 18-35 years then you may be eligible to participate.**
- **Total time commitment will be approximately 20 hours over a period of 1-2 weeks.**

Participation will involve:

- **performing a strenuous exercise protocol with the right calf muscle designed to elicit muscle soreness;**
- **completing a number of strength tests of the right calf muscle;**
- **completing questionnaires designed to assess intensity of muscle soreness;**
- **undergoing three one hour treatments of hyperbaric oxygen (or a placebo treatment) at the Misericordia Hospital, Edmonton;**
- **undergoing a number of imaging/spectroscopy sessions within a magnetic resonance imaging device at the University of Alberta.**

If you are interested in finding out more about this study then please contact Tony Webster at 436-2184 or by e-mail: awebster@gpu.srv.ualberta.ca

Appendix D:

EFFECTS OF HYPERBARIC OXYGEN ON RECOVERY FROM EXERCISE-INDUCED MUSCLE DAMAGE.

Please tick yes or no after each statement:

1. Has your right calf muscle ever been severely injured?
   Yes____No____

2. Are you currently taking or have you recently taken any kind of anti-inflammatory drug?
   Yes____No____

3. In the previous 3 months, have you experienced muscle soreness within the right calf muscle?
   Yes____No____

4. In the previous 3 months, have you performed weight training of any kind on the right calf muscle (eg. standing or seated calf raises)?
   Yes____No____

I have read, understood and completed this questionnaire.

Signature:_____ Date:_____

Witness:_____ Date:_____

Appendix E:

EFFECTS OF HYPERBARIC OXYGEN ON RECOVERY FROM EXERCISE-INDUCED MUSCLE DAMAGE.

Please tick yes or no after each statement:

1. Are you currently suffering from any form of infection (*ie*. a cold, fever, or any kind of viral/bacterial infection)?
   Yes____No____
2. Are you presently experiencing, or do you routinely experience, problems with your sinus cavities (*ie*. blocked sinuses, etc.)?
   Yes____No____
3. Do you possess any kind of seizure disorder (eg. epilepsy) or have you ever experienced a seizure?
   Yes____No____
4. Do you have a lung problem such as emphysema or severe asthma?
   Yes____No____
5. Do you have a history of thoracic or ear surgery?
   Yes____No____
6. Do you have any kind of malignant disease (eg. cancer)?
   Yes____No____
7. Do you experience, or have you experienced in the past, any serious eye problems (eg. optic neuritis, detached retina, etc.)?
   Yes____No____
8. Are you at all claustrophobic?
   Yes____No____
9. Are you currently taking medication/drugs of any sort on a regular basis?
   Yes____No____
   If "Yes", please expand:_____

If there are any changes in your status relative to the above questions, please bring this information to the attention of one of the project investigators.

I have read, understood and completed this questionnaire.

Signature:_____ Date:_____

Witness:_____ Date:_____

Appendix F:

CONSENT FORM FOR NORMAL VOLUNTEERS TO BE EXAMINED BY IN-VIVO NUCLEAR MAGNETIC RESONANCE (NMR)

Peter S. Allen, Dept. of Biomedical Engineering

I understand that in order to develop and perfect the application of Nuclear Magnetic Resonance (NMR) to the solution of medical problems, it is necessary to carry out development work while using normal volunteers as subjects. I have been assured that all information obtained will be kept confidential and I agree to spend up to 2 hours of my time to help with this investigation.

I understand that there are no known harmful effects of this technique when operated under conditions that are within the limits set by Federal regulation. I understand that the examination will be entirely non-invasive and painless. I have been warned to keep my eyes closed during the laser positioning part of the procedure and I agree to do so.

The dangers presented by ferromagnetic objects (any material that can be magnetised) when exposed to large magnetic fields have been made clear to me, and I can state categorically that:

(Please answer True or False, T/F)

_____ I have removed my hearing aid and false teeth, if I wear either.

_____ I do not wear a cardiac pacemaker, implanted neurostimulator, or implanted drug delivery system.

_____ I have not undergone any form of surgery within the last two months.

_____ I have never undergone a cardiac valve replacement.

_____ I have never had an operation on my head and can therefore exclude the possibility of metal clips adjacent to or within my brain.

_____ I have never had an operation on my chest and can therefore exclude the possibility of metal clips adjacent to or within my heart.

_____ I have never had any form of vascular surgery and can therefore exclude the possibility of metal clips attached to my vascular system.

_____ I have never had a bone fracture and can therefore exclude the possibility of metal pins and screws supporting my skeletal system.

_____ I have never undergone a joint replacement.

_____ I have never been injured by a metallic foreign object which was not subsequently removed.

_____ I have never worked as a welder, lathe operator or sheet metal worker or in a similar trade.

_____ I am not wearing an IUD contraceptive device.

_____ There is no possibility that I am pregnant.

The points presented above have been explained to me. I have also been given the opportunity to ask questions, and the questions I have asked have been answered to my satisfaction. In view of this understanding and being under no duress or compulsion, I volunteer freely and willingly to undergo an NMR examination and fully accept responsibility for my actions. I have been given a copy of this consent form.

Name (please print).	Signature.	Date.
Project name (print).	Consent obtained by (print and sign).	

Appendix G:

EFFECTS OF HYPERBARIC OXYGEN ON RECOVERY FROM EXERCISE-INDUCED MUSCLE DAMAGE.

Participant Information Letter

Principal Investigator:	Dr. D. Syrotuik	Physical Education and Recreation	492-1018
Co-Investigator(s):	A.L. Webster	Physical Education and Recreation	436-2184
	Dr. G. Bell	Physical Education and Recreation	492-2018
	Dr. R. Jones	Pulmonary Medicine	492-7144
	Dr. B. Fisher	Physical Education and Recreation	492-8273
	Dr. P. Allen	Biomedical Engineering	492-6397
	Dr. M. Young	Emergency Care Physician, Misericordia Hospital.	930-5717

Purpose of this study:

The purpose of this study will be to investigate the effect(s) of hyperbaric oxygen therapy on recovery from muscle damage and muscle soreness that has been caused by prior strenuous exercise in the calf muscle. This study is being performed as part of the Ph.D. degree of one of the co-investigators (A.L. Webster).

Background:

In recent years numerous professional sports teams have purchased hyperbaric oxygen chambers in the belief that hyperbaric oxygen (HBO) therapy will help athletes recover more quickly from injury. However, there is currently little convincing scientific evidence to suggest that HBO is effective for this purpose. This study has been designed to help answer the question as to whether HBO is useful in the recovery from muscular injury. Male subjects 18-35 years of age are being recruited for participation in this study.

Procedures:

As a participant your time commitment in this study will be about 15-20 hours total over a period of 8 days. You will be subjected to a number of different tests which will take anywhere from ½-1½ hour(s) each (they are described in more detail below). As stated above, the main purpose of the study will be to investigate if HBO therapy will help your damaged/sore calf muscle to recover more quickly.

Prior to causing soreness/damage in your right calf muscle, you will need to undergo a number of tests designed to assess the function and appearance of your calf muscle in an undamaged state. These tests are called "baseline" tests. You will need to visit the Faculty of Physical Education and Recreation at the University of Alberta for two baseline tests (on separate days) of the maximal strength and endurance of your right calf muscle. These tests will require you to be strapped into a machine called an "isokinetic dynamometer" such that your calf muscle can be isolated and its strength/endurance examined. Immediately prior to these three tests, you will be instructed on how to correctly fill out a questionnaire designed to assess pain levels. As you will not be experiencing pain or discomfort at that time you will be provided with an imaginary painful scenario. Your ability to respond correctly to the questionnaire will be assessed and will be of importance later on in the study.

You will also be required to undergo one baseline test within a magnetic resonance imaging (MRI) machine on the University of Alberta campus. This test will take approximately one hour and you will simply be required to lie inside the machine while we take images and measurements of your right calf muscle.

After all the baseline tests are completed (on a subsequent day) you will be required to report to the Faculty of Physical Education and Recreation where you will undergo a strength test on a weight machine designed to determine the maximal amount of weight that you can lift once with your calf muscles only. Immediately after this test you will be required to perform an exercise protocol (again only with the calf) that will be designed to cause you to experience muscle soreness over the next few days.

Between two to four hours after the exercise protocol, you will be required to report to the Misericordia Hospital, Edmonton for your first session within a hyperbaric chamber. You will simply be required to relax and lie inside the chamber for approximately 1½ hours while breathing a gas mixture under pressure. Subjects will be exposed to either of two gas mixtures: 100% oxygen or a mixture equivalent to normal atmospheric oxygen concentration. Neither the subjects nor the investigators will know which gas mixture you are breathing - you have an equal chance of being assigned to the group breathing 100% oxygen or the group breathing the "normal" oxygen mixture. [If necessary, the principal investigator could determine the nature of the treatment administered in a timely fashion]. You will be required to report to the Misericordia Hospital for two more sessions within the hyperbaric chamber 24 hours and 48 hours after the first treatment.

Isokinetic strength testing and pain questionnaire measurements will be repeated exactly as for the baseline testing (above) on the first day *after* the exercise protocol, the second day, the third day and the fifth day after. You will be required to undergo repeat MRI tests on the first day, the third day and the fifth day after the exercise protocol. The locations and timing of these tests will be the same as for the baseline tests.

Benefits:

The results of this study will be of no direct benefit to you but the information obtained may help to reveal whether hyperbaric oxygen therapy has a role to play in the recovery from muscle injury.

Risks:

You should expect to experience pain/discomfort in your calf muscles as a result of the exercise protocol that you will perform. It is difficult to predict how much discomfort you will feel and for how long - there are large differences between individuals. However, the effects are fully repairable and muscle soreness should disappear within a week. If you take any kind of medication to relieve inflammation or discomfort in the calf muscle you will be required to withdraw from the study.

Hyperbaric oxygen therapy will be performed at the Misericordia Hospital, Edmonton by highly trained hyperbaric technicians. However, there are a number of risks associated with hyperbaric oxygen (HBO) therapy. You will not be allowed to undergo hyperbaric oxygen therapy if you have any of the following conditions: upper respiratory tract infections, seizure disorders (such as epilepsy), emphysema, severe asthma, pulmonary lesions, a history of thoracic or ear surgery, uncontrolled high fever, malignant disease and optic neuritis. You will be required to fill out a questionnaire designed to determine if you have any of these conditions and you will also undergo a medical examination by a physician.

The most common complications associated with HBO involve trauma of air filled body cavities due to changes in external pressure. For example, pain may arise as a result of blocked sinuses or from an inability to equalize middle ear pressure with that of outside air during compression. However, prior studies have indicated that the chances of a healthy subject experiencing problems with cavity trauma are around 1 in 300.

Oxygen at high concentrations can be toxic to the body if the body is exposed for prolonged periods. Central nervous system oxygen toxicity is a problem mainly associated with prolonged HBO treatment sessions at relatively high pressures. At the pressures being used in the present study, it has been estimated that the chance of experiencing oxygen-induced seizures is approximately 1 in 10,000. In the unlikely event of a subject experiencing oxygen toxicity, the fraction of inspired oxygen will be decreased to normal levels (*ie.* 21% of inspired air) by asking the subject to breathe through an air mask. This procedure is sufficient to cause symptoms to resolve and they will generally not recur if 100% oxygen is restarted. Even if a subject does experience oxygen toxicity, there are no lasting side effects.

Claustrophobia is sometimes observed within hyperbaric chambers and/or magnetic resonance imaging machines. If subjects become claustrophobic, they will be removed from the chamber/machine.

Confidentiality:

All study data will remain in the possession of A.L. Webster (co-investigator) to ensure confidentiality and anonymity. Data will be stored within locked filing cabinets. Only the principal investigator and co-investigators will have access to the data. Participants will not be identified in any presentation or publication of this data.

Freedom to withdraw:

You are free to withdraw from the study at any time from the study should you so wish without any adverse consequences. You do not have to give a reason for withdrawing.

Additional Contacts:

If you have any concerns about any aspect of this study, you may contact the Patient Concerns Office of the Capital Health Authority at 474-8892. This office has no affiliation with study investigators.

Appendix H:

INFORMED CONSENT FOR PARTICIPANTS

Title of Project: Effects of hyperbaric oxygen on recovery from exercise-induced muscle damage.

Principal Investigator:	Dr. D.G Syrotuik	Physical Education and Recreation	492-1018
Co-Investigator(s):	A.L. Webster	Physical Education and Recreation	436-2184
	Dr. G.J. Bell	Physical Education and Recreation	492-2018
	Dr. R. Jones	Pulmonary Medicine	492-7144
	Dr. B. Fisher	Physical Education and Recreation	492-8273
	Dr. P. Allen	Biomedical Engineering	492-6397
	Dr. M. Young	Emergency Care Physician, Misericordia Hospital.	930-5717

Please complete the following:

Do you understand that you have been asked to be in a research study?	Yes	No
Have you read and received a copy of the attached Information Sheet?	Yes	No
Do you understand the benefits and risks involved in taking part in this research study?	Yes	No
Have you had an opportunity to ask questions and discuss this study?	Yes	No
Do you understand that you are free to refuse to participate or withdraw from the study at any time? You do not have to give a reason and it will not affect you in any way.	Yes	No
Has the issue of confidentiality been explained to you? Do you understand who will have access to your results?	Yes	No

This study was explained to me by: _____

I agree to take part in this study.

_____	_____	_____
Signature of Research Participant	Date	Witness
_____		_____
Printed Name		Printed Name

I believe that the person signing this form understands what is involved in the study and voluntarily agrees to participate.

_____	_____
Signature of Investigator or Designee	Date

Appendix I:

LITERATURE REVIEW OF THE CAUSES AND CONSEQUENCES OF EXERCISE-INDUCED MUSCLE DAMAGE

Exercise-induced muscle damage (EIMD) typically occurs after performance of unaccustomed exercise, particularly exercise involving active lengthening of muscles, *ie.* eccentric contractions (Armstrong *et al.*, 1991; Ebbeling & Clarkson, 1989). Evidence of damage of this nature to muscles includes morphological disruptions of muscular structure, delayed onset muscular soreness and pain, performance decrements and elevation of muscle protein levels in the blood (Ebbeling & Clarkson, 1989; Kuipers, 1994). It is clear that muscles are not permanently impaired by this kind of insult and, in fact, some have suggested that EIMD is a normal precursor of muscle adaptation to increased use (Armstrong *et al.*, 1991). Moreover, it has been widely demonstrated that muscles adapt to the stress of repeated exercise such that subsequent bouts of eccentric activity result in less damage (Ebbeling & Clarkson, 1989). In spite of the frequent occurrence of this form of injury, and its practical consequences, surprisingly little is known about the causative factors or the cellular mechanisms involved.

Interpretation of the results of studies performed in the area of EIMD is complicated by the fact that both animal and human models have been utilized and the protocols used to elicit muscle damage have varied widely. With animal models of EIMD, some investigators have investigated isolated muscles in vitro, some have used a combined in vitro/in situ muscle model and yet others have taken a "whole body" approach by subjecting animals to downhill running. With human models, examples of protocols used to elicit muscle damage include downhill running, step tests, eccentric bicycle ergometer exercise and eccentric exercise on isolated muscle groups (such as the elbow flexors or knee extensors). Also, many studies (animal and human) have used both males and females without accounting for the fact that responses to an identical bout of exercise designed to elicit EIMD may be quite different between the sexes (Bär *et al.*, 1988).

(2.1) Initial causes of exercise-induced muscle damage.

(2.1.1) Mechanisms of exercise-induced muscle damage.

Two basic mechanisms have been proposed to explain how exercise may initiate EIMD: metabolic and mechanical (Ebbeling & Clarkson, 1989). Possible metabolic mechanisms that have been proposed to cause EIMD include insufficient mitochondrial respiration (as a result of ischaemia or hypoxia in muscle), higher temperatures, oxygen free radical production, reduced pH and accumulation of waste products (such as lactic acid) in muscle (DeVries, 1966; Kuipers, 1994; Armstrong *et al.*, 1991; Ebbeling & Clarkson, 1989). The mechanical mechanism is based on the belief that eccentric contractions, as opposed to concentric or isometric contractions, cause greater tensions to be generated in skeletal muscle which overwhelms the muscle's structural limits and result in damage (Kuipers, 1994; Armstrong *et al.*, 1991; Ebbeling & Clarkson, 1989).

Most investigators now believe that mechanical, rather than metabolic, factors are the major cause of EIMD after eccentrically biased activities (Fridén & Lieber, 1992; Kuipers, 1994; Armstrong *et al.*, 1991; Ebbeling & Clarkson, 1989). The major problem with all metabolic hypotheses is that eccentric contractions of muscle have consistently been shown to cause greater damage to skeletal muscle than concentric and isometric contractions, despite the fact that eccentric contractions are metabolically "cheaper" (Armstrong *et al.*, 1991). If metabolic mechanisms were the primary initiators of EIMD then one would expect that concentric contractions, which cause a greater metabolic "stress" on the muscle, would result in greater damage.

Metabolic mechanisms, however, do play a role in the phenomenon of EIMD. After initial mechanical damage to muscle, a number of metabolic mechanisms operate first to amplify the damage and ultimately to initiate healing and regeneration of muscle. In particular, Armstrong (1990) has presented convincing evidence that an imbalance in calcium concentration in muscle cells, caused by initial mechanical damage to muscle fibres, plays a central role in the autogenetic phase of EIMD (see below). Thus, an initial mechanical injury followed by a secondary biochemical injury are likely responsible for

the changes in muscle following eccentric exercise (MacIntyre *et al.*, 1995; Faulkner *et al.*, 1993).

(2.1.2) Characteristics of lengthening contractions associated with EIMD.

Studies using electromyography (EMG) have shown that, at a given submaximal force or power output, EMG activity in muscle is lower during negative (eccentric) work than concentric work (Armstrong *et al.*, 1991; Ebbeling & Clarkson, 1989). This suggested that relatively fewer motor units were recruited at the same workload during eccentric contractions resulting in greater tension per cross-sectional area of active muscle. Faulkner *et al.* (1993) have stated that the force developed by a fully activated muscle during lengthening contractions was approximately twofold greater than that developed during a maximum isometric tetanic contraction. However, the total number of cross-bridges attached in a strongly bound state during lengthening was only about 10% greater than the number attached during an isometric contraction (Faulkner *et al.*, 1993). This translated into increased mechanical stress on individual cross-bridges. It has been widely suggested that these high tensions cause muscle injury (McCully & Faulkner, 1986; Armstrong *et al.*, 1991; Ebbeling & Clarkson, 1989).

More recent evidence has suggested that muscle damage is not a function of absolute force or tension generated in muscle but the magnitude of strain during active lengthening (Lieber & Fridén, 1993). These authors determined that active strain during eccentric work was related to the speed at which a muscle was lengthened while it was contracting. It was suggested that the greater damage elicited by faster eccentric contractions was due to the fact that cross-bridge cycling could not keep pace with the change in length of the muscle (McCully & Faulkner, 1986).

McCully & Faulkner (1986) also showed that the extent of muscle injury sustained as a result of lengthening contractions increased with increasing durations of eccentric work up to a certain point in time. They found that, after five minutes of lengthening contractions, fatigue of muscle fibres appeared to confer some degree of protection against injury. They also demonstrated that muscles fatigued previously by isometric contractions were relatively resistant to injury by a subsequent protocol of

lengthening contractions. The authors hypothesized that fatigue reduced the ability of muscle to generate high forces and thus prevented further damage from occurring.

Newham *et al.* (1988) and Child *et al.* (1998) have demonstrated that there is a length-dependent component in the development of pain and fatigue after eccentric exercise. These investigators found that greater injury occurred when eccentric contractions were initiated at longer muscle lengths. These findings were supported by Faulkner *et al.* (1993) who showed that damage was greater in muscles that were subjected to lengthening contractions beyond the optimum length of fibres for force development (L_f). The magnitude of the decrease in force after such contractions was partially correlated with both the average force developed during the contraction and the displacement beyond L_f. The product of the average force and displacement (work done to stretch the active muscle) explained 87% of the variation in the decrease in force (Faulkner *et al.*, 1993).

Therefore it is now apparent that high force generation alone in muscle may not be the complete explanation for damage after eccentric exercise. A number of other characteristics of lengthening contractions and the state of the muscle have an influence on the amount of injury elicited.

(2.1.3) Location of initial damage to skeletal muscle.

Evidence of structural damage has been observed immediately post insult in both animal and human studies (Fridén & Lieber, 1992; Newham *et al.*, 1983b) and the extent and severity of the morphological evidence of injury typically worsen over the next 1 to 3 days - termed "secondary injury" (Faulkner *et al.*, 1993; Newham *et al.*, 1983b). Immediate evidence of damage is most easily seen in the form of Z-band streaming/smearing, A-band disruption and misalignment of myofibrils (Fridén & Lieber, 1992). However sarcolemmal disruption, sarcotubular swelling/disruption, cytoskeletal damage and extracellular matrix abnormalities have also been reported (Fridén & Lieber, 1992; Byrd, 1992). The precise location of the initial mechanical damage to muscle is subject to controversy. However, in recent years evidence has emerged to suggest that the cytoskeleton, which has both exosarcomeric and

endosarcomeric components, may be the first structure to yield during eccentric exercise (Waterman-Storer, 1991). Injury may occur to the exosarcomeric skeleton as a result of cytoskeletal damage to intermediate filaments (composed largely of desmin but also vimentin and synemin) which may cause the Z-lines to "stream" (Waterman-Storer, 1991). Damage to the endosarcomeric cytoskeleton, composed largely of the proteins titin and nebulin, has been implicated in myosin filament decentralization within the sarcomere (Waterman-Storer, 1991). Lieber & Fridén (1993) have proposed that excessive active strain exceeds the limits of the exosarcomeric cytoskeletal framework which joins the cytoskeleton to the muscle cell basal lamina and sarcolemma. Disruption of this framework, which consists of talin, veniculin and α-actinin, among other proteins, and the integrin family of adhesion receptors, may also result in cytoskeletal damage.

There has been discrepancy with regards to which classes of muscle fibre (I, IIa or IIb) are more susceptible to injury, especially when comparing animal to human models. Fridén & Lieber (1992) have argued that type I fibres are less vulnerable to repetitive, high tension stress than type II fibres due to their broader Z-bands and the fact that they have a higher oxidative capacity and are less prone to fatigue. They have proposed that the relatively rapid fatiguability of type II fibres would lead them to enter a rigor or high stiffness state, thus rendering them more susceptible to mechanical disruption. However, the results of McCully & Faulkner (1986) implied that muscle fibres that fatigued most rapidly (*ie*. type II fibres) were protected from EIMD. Also, the results of Mair *et al.* (1992, 1995) have provided evidence of type I (slow twitch) fibre injury on account of the increased levels of slow twitch skeletal muscle myosin heavy chains found in plasma after lengthening contractions of the quadriceps muscle group. Therefore, there is evidence that both fast and slow twitch fibres may be injured (Ebbeling & Clarkson, 1989). Selective damage is likely related to intensity and duration of eccentric exercise, motor unit recruitment patterns and/or structural differences between type I and II fibres.

It is often difficult to assess the exact location and extent of damage within muscle fibres because of the small specimen size and physiological states of sectioned fibres. However, it appears that exercise-induced injury is restricted to relatively short segments of few fibres and that sections of focal necrosis can be "walled-off" from the remaining fibre and then repaired (Armstrong *et al.*, 1983; Ebbeling & Clarkson, 1989).

(2.2) Consequences of exercise-induced muscle damage.

(2.2.1) Loss of muscle function

It has been well documented that high intensity eccentric exercise causes a dramatic loss of eccentric, isometric and concentric strength which is most evident immediately after cessation of the exercise bout (Clarkson *et al.*, 1992; MacIntyre *et al.*, 1996). There is rapid partial recovery of strength within hours of the cessation of exercise which is most likely due to recovery from fatigue (Clarkson *et al.*, 1992; Faulkner *et al.*, 1993). After this, strength is slowly restored such that by 10-12 days after exercise a deficit may still remain (Clarkson *et al.*, 1992; Cleak & Eston, 1992). Newham *et al.* (1983a, 1987, 1988) showed that eccentrically damaged muscles exhibited a force-frequency curve that was shifted to the right (*ie.* reduced ability to generate force when stimulated at low frequencies). This characteristic was found to persist for at least four days and was attributed to damaged sarcoplasmic reticulum within muscle fibres (Newham *et al.*, 1983a). Some investigators have observed a bimodal pattern in the recovery of eccentric torque (MacIntyre *et al.*, 1996; Faulkner *et al.*, 1993) where the initial recovery of muscle force post-exercise was followed by a second decline in muscle force between one and two days post-exercise. The second decline in muscle force was attributed to worsening damage within muscle as a result of phagocytic activity at the site of initial lesions.

The exact cause of the decline in strength after eccentric exercise is unclear. It is possible that myofibrils damaged by eccentric exercise would result in the strength decrements observed. However, ultrastructural damage has been shown to become worse in the days following exercise, during the time that strength was recovering (Clarkson *et al.*, 1992; Newham *et al.*, 1983b). Armstrong (1984) suggested that delayed onset muscular soreness and pain prevented the subject from voluntarily producing maximal force. However, this is unlikely as it has been found that the time course of soreness development and strength loss/recovery are very different (Clarkson *et al.*, 1992). A further convincing line of evidence refuting that soreness was the cause of reduced strength was presented by Newham and colleagues (1987). These investigators

demonstrated that bypassing voluntary effort with superimposed electrical stimulation of sore muscles did not result in an increase in maximal isometric force.

Understanding of the loss of muscle function after eccentric exercise has been complicated by the fact that one cannot discount an involvement of the nervous system in attenuating the strength loss and facilitating recovery (Clarkson *et al.*, 1992). It is possible that neural activation patterns could change such that the more severely damaged fibres could be "bypassed". Newham *et al.* (1983a) have presented evidence to suggest that electromyographic patterns are altered after eccentric exercise.

Saxton *et al.* (1995) confirmed that eccentric exercise resulted in numerous indices of neuromuscular dysfunction. They determined that tremor amplitude of the involved muscle group was increased until 48h after exercise. There was also a loss in proprioceptive function as perception of joint angle and force were significantly impaired during the days following the damaging exercise. Similar results were obtained by Pearce *et al.* (1998) who determined that performance of the biceps brachii during a visuomotor tracking pursuit task was significantly impaired for several days following eccentric exercise. These studies reveal clear implications for individuals who may be required to perform fine motor tasks during the days subsequent to muscle damage.

Muscle dysfunction after eccentric exercise has also been monitored by the measurement of relaxed and flexed arm angles (Clarkson & Tremblay, 1988; Cleak & Eston, 1992; Ebbeling & Clarkson, 1990; Nosaka *et al.*, 1991; Rodenburg *et al.*, 1993; Rodenburg *et al.*, 1994a; Saxton *et al.*, 1994; Stauber *et al.*, 1990). Typically, immediately after eccentric exercise of the elbow flexor muscles (biceps brachii and brachialis) a decrease has been seen in relaxed elbow angle and an increase in flexed elbow angle. These features have usually persisted for several days after the exercise bout. A decrease in relaxed elbow angle indicated a relative muscle contracture of the elbow flexors at rest and has been attributed either to increased swelling in muscle (Stauber *et al.*, 1990) or to an abnormal accumulation of free calcium in the sarcoplasm of the muscle fibres (Cleak & Eston, 1992; Nosaka *et al.*, 1991; Clarkson & Tremblay, 1988). An increase in flexed elbow angle indicated an impaired contractile function and has been attributed to altered myofibrillar function as a result of eccentric damage (Nosaka *et al.*, 1991).

It also appears that exercise-induced muscle damage has the ability to impair the function of sarcolemmal transporter proteins such as GLUT-4 (Asp *et al.*, 1995). This might explain why glycogen resynthesis is decreased after eccentric exercise (Costill *et al.*, 1990).

(2.2.2) Release of muscle proteins into blood.

Muscle damage can be measured either directly (by the presence of ultrastructural damage demonstrated by electron microscopy) or indirectly. The most widely used indirect indicators of muscle damage has been the appearance of proteins, resident within skeletal muscle, in the blood (Ebbeling & Clarkson, 1989; Faulkner *et al.*, 1993). Examples of such proteins include creatine kinase (Clarkson & Tremblay, 1988; Ebbeling & Clarkson, 1990; Van Der Meulen *et al.*, 1991; Fridén *et al.*, 1989; Evans *et al.*, 1986; Paul *et al.*, 1989; Saxton *et al.*, 1994; Rodenburg *et al.*, 1993; Mair *et al.*, 1992; Mair *et al.*, 1995), lactate dehydrogenase (Van Der Meulen *et al.*, 1991; Fridén *et al.*, 1989; Armstrong *et al.*, 1983), aspartate aminotransferase (Van Der Meulen *et al.*, 1991; Nørregaard *et al.*, 1982; Janssen *et al.*, 1989; Noakes & Carter, 1982), myoglobin (Paul *et al.*, 1989; Rodenburg *et al.*, 1993; Nørregaard *et al.*, 1982; Cummins *et al.*, 1987; Mair *et al.*, 1992), 3-methylhistidine (Evans *et al.*, 1986; Paul *et al.*, 1989), alkaline phosphatase (Nørregaard *et al.*, 1982; Lijnen *et al.*, 1988; Noakes & Carter, 1982), glutamic oxaloacetic transaminase (Fridén *et al.*, 1989; Lijnen *et al.*, 1988), aldolase (Lijnen *et al.*, 1988), glutamic pyruvic transaminase (Lijnen *et al.*, 1988), alanine aminotransferase (Janssen *et al.*, 1989), myosin heavy chains (Mair *et al.*, 1992; Mair *et al.*, 1995) and skeletal troponin I (Sorichter *et al.*, 1997). In a healthy undamaged muscle, the sarcolemma surrounding muscle fibres is impermeable to these proteins. However, after damaging eccentric exercise, it has been proposed that the sarcolemmal barrier is compromised and muscle proteins subsequently appear in the bloodstream (Ebbeling & Clarkson, 1989).

By far the most frequently used plasma indicator of muscle damage has been creatine kinase (CK) (Clarkson *et al.*, 1992). CK exists as three different isoenzymes that are specific to different types of tissue: CK-MM (found in skeletal muscle), CK-MB

(found in cardiac and skeletal muscle) and CK-BB (found in brain) (Ebbeling & Clarkson, 1989). CK-MM is primarily responsible for the postexercise rise in plasma CK activity as it accounts for 90-100% of the total CK activity in skeletal muscle (Rogers *et al.*, 1985).

There are a number of significant drawbacks to using CK as a marker of muscle damage which have often been overlooked by investigators. First, total CK concentration in the blood represents a balance of efflux from skeletal muscle and removal by the liver and hence peak changes in CK should be interpreted with caution (Ebbeling & Clarkson, 1989; Evans & Cannon, 1991). Second, damage to muscle fibres is not necessarily reflected in proportional increases in plasma CK activity (Kuipers, 1994; Van der Meulen, 1991; Janssen *et al.*, 1989). Many investigators have assumed that higher CK responses to exercise indicated greater damage to skeletal muscle but this is probably only partially true. Van der Meulen (1991) have obtained convincing evidence that the use of plasma enzyme activities for estimating the amount of muscle damage should be cautioned. Third, the plasma CK response after a similar bout of exercise has been shown to differ widely between individuals. This unexplained phenomenon has led Clarkson & Ebbeling (1988) to discriminate between "no", "low" and "high" responders. These authors also concluded that the increase in circulating CK was unrelated to either the development of muscle soreness, the amount of strength loss after exercise, fitness level of the subject or lean body weight. Finally, it has been demonstrated that females exhibit a lower CK response to a similar bout of exercise than males and that this response is likely due to some protective effect of oestradiol on the muscle cell membrane (Bär *et al.*, 1988). Van der Meulen (1991) determined that, after a similar running protocol, higher CK release was noted in male rats compared to female rats despite the fact that there were no differences between the sexes in the extent of histological damage observed.

The amount of change in serum CK and the time course of these changes depends largely on the kind of exercise performed. For example, it has been found that the increase in serum CK activity after downhill running was substantially lower and the peak activity occurred earlier (about at 24 hours post-exercise) than that seen after isolated high-force eccentric exercise (2-7 days post-exercise) (Clarkson *et al.*, 1992;

Ebbeling & Clarkson, 1989). It is likely that the peak CK activity found after exercise is, at least to some degree, related to the amount of damage incurred but it is not clear why there are different time courses of CK responses after different exercise modes.

(2.2.3) Loss of calcium homeostasis in muscle fibres

Armstrong *et al.* (1991) have presented a strong body of collective evidence to suggest that loss of Ca^{2+} homeostasis within muscle fibres plays a pivotal role in the pathology associated with EIMD. However, they do concede that the evidence suggesting an involvement of Ca^{2+} in EIMD is largely associative and that a causal link has not yet been established. They have proposed a sequence of events or phases in muscle that occurs as a result of damage induced by exercise. They hypothesized that initial mechanical damage (most likely of a mechanical nature) suffered by muscle fibres is followed by a "Ca^{2+} overload phase". There are two potential sources of Ca^{2+}: extracellular and intracellular. Extracellular Ca^{2+} may be allowed to leak into the cell down a strong electrochemical gradient as a result of mechanical disruption of the sarcolemma. Alternatively, disruption of the sarcoplasmic reticular membrane or dysfunction of the sarcoplasmic reticular Ca^{2+}-ATPase may result in Ca^{2+} accumulation in the sarcoplasm (Byrd, 1992).

If the cell mechanisms for buffering and translocation of Ca^{2+} are overwhelmed in the face of elevated intracellular Ca^{2+}, then Armstrong *et al.* (1991) have hypothesized that injury becomes "irreversible" as a result of activation of several intrinsic degradative pathways in the fibres. This signifies the start of the "autogenetic" phase during which a number of cellular structures are degraded (MacIntyre *et al.*, 1995). Enzymes that are activated by Ca^{2+} during this phase include phospholipase A_2 (which produces arachidonic acid, prostaglandins and leukotrienes, amongst other eicosanoid substances), calpains (calcium activated neutral proteases) and lysosomal proteases. In addition, elevated intracellular Ca^{2+} can disrupt normal mitochondrial function and cause sarcomere contracture (Armstrong *et al.*, 1991). There is strong evidence to suggest that these processes contribute to an amplification of the initial mechanical damage to the muscle fibre, termed secondary injury (Belcastro, 1993; Armstrong *et al.*, 1991).

By 2 to 6 hours after initiation of injury, an inflammatory "phagocytic" phase is in evidence in the muscle which is essential for the removal of injured tissue and for stimulating regeneration of the damaged fibres (Armstrong *et al.*, 1991). Calcium may play a prominent role in this phase also. Smith *et al.* (1993) have hypothesized that elevated Ca^{2+} stimulates macrophages (the predominant inflammatory cell type found in damaged muscle) to synthesize prostaglandin E_2 which in turn may be involved in the production of delayed-onset muscle soreness. A more detailed discussion of role of inflammation in muscle soreness and EIMD is found below. Finally, 4 to 5 days after injury there is evidence of the beginning of the "regenerative" phase, during which the muscle undergoes complete regeneration.

(2.2.4) Delayed-onset muscle soreness (DOMS).

Delayed-onset muscle soreness has been defined as a dull, aching pain combined with tenderness and stiffness that usually develops during the first 8 to 48 hours following unaccustomed eccentric exercise and peaks between 24 and 72 hours (Ebbeling & Clarkson, 1989). It then subsides and disappears within 5 to 7 days of exercise (Ebbeling & Clarkson, 1989). Despite the widespread occurrence of muscle soreness, the specific etiology is not completely understood. Stauber and colleagues (1990) have suggested that DOMS is due to a complex set of reactions involving disruption of connective tissue and muscle fibres. Smith (1991) has presented evidence to suggest that acute inflammation is the underlying cause of DOMS.

The study of DOMS is beset with a number of problems, the most obvious being that it cannot be studied in the animal model. In humans, reliable and valid measurement of DOMS presents some major difficulties, largely due to the fact that an individual's sensory perception of DOMS is modulated by past experience where attitudes and psychological variables may influence description of the sensation (MacIntyre *et al.*, 1995). In a recent review of the topic, MacIntyre *et al.* (1995) have suggested that tools used to assess muscle soreness should measure not only the intensity of the sensation but also the affective, or emotional, response to the pain. These authors suggested that the Descriptor Differential Scale (DDS) (Gracely & Kwilosz, 1988) and the McGill Pain

Questionnaire (Melzack, 1975) were examples of instruments that fulfilled these criteria. However, only one study could be found in the literature concerned with DOMS that has utilized an instrument of this kind. MacIntyre *et al.* (1996) successfully used the DDS to monitor DOMS after high force eccentric contractions of the quadriceps muscle. This instrument has been validated (Doctor *et al.*, 1995) and found to satisfy standard psychometric criteria for reliability, objectivity and item homogeneity (Gracely & Kwilosz, 1988).

Investigators have used either of two methods to gauge the intensity of pain: a questionnaire or a rounded wooden probe connected to a strain gauge. The questionnaires most commonly used have been those where perceived soreness is rated on a verbally anchored, fixed ordinal scale, usually from 0 to 10 (Clarkson *et al.*, 1992; Clarkson & Tremblay, 1988; Ebbeling & Clarkson, 1990; Mair *et al.*, 1992; Mair *et al.*, 1995; Nosaka *et al.*, 1991; Rodenburg *et al.*, 1993; Stauber *et al.*, 1990). As well as suffering from the limitation outlined above, these methods may be insensitive and susceptible to scaling error when used for repeat testing. In other words, subjects may repeatedly use the same category or base their rating on a past specific response (MacIntyre *et al.*, 1995). Instruments that collect multiple responses (such as the DDS) minimize this problem and are potentially more sensitive to changes in pain sensation. Use of a wooden probe to assess the degree of DOMS involves applying it to specific areas on the tender muscle and gradually increasing the force until the subject first indicates that they feel tenderness (Newham *et al.*, 1983a; Newham *et al.*, 1988). The smaller the force required to elicit tenderness, the more severe the DOMS is assumed to be.

Studies have often used isolated muscle biopsy samples to examine the relationship between muscle injury and DOMS post-exercise (Newham *et al.*, 1983b). However, muscle biopsies are restricted to a small and specific location of the muscle and there is usually a limited number that may be obtained from each subject (MacIntyre *et al.*, 1996). Also, studies investigating the relationship between muscle damage and DOMS have often relied upon the sensation of soreness to "guide" a biopsy procedure, under the assumption that the most damage will be found in this area. However, Nurenburg *et al.* (1992) have shown, using magnetic resonance imaging (MRI), that the

areas of muscle that are most sore are not necessarily those where the most damage has occurred. These authors encouraged the use of MRI to guide muscle biopsy procedures.

Understanding of the etiology of DOMS has been complicated by the observation that its time course does not correlate well with that of ultrastructural muscle damage (Newham *et al.*, 1988; Clarkson *et al.*, 1992; Mair *et al.*, 1992). Also, it is feasible that the etiology of DOMS may be different depending on the type of exercise protocol that has been used to elicit muscle damage. For example, downhill running involves a greater metabolic component than isolated high-force eccentric exercise and hence the causes of DOMS may be subtly different between these two modes of exercise. The reader should be cautious when comparing studies that have used different exercise protocols to elicit muscle damage.

The precise cause of DOMS remains unresolved. It is known that the generation of painful sensations involves activation of pain afferents, particularly group III and IV fibres (Smith, 1991; Ebbeling & Clarkson, 1989; MacIntyre *et al.*, 1996). These are found throughout muscle but are particularly dense within the connective tissue of the muscle (Ebbeling & Clarkson, 1989; MacIntyre *et al.*, 1996). Since group IV fibres carry dull, diffuse pain and are twice as common as group III fibres, Armstrong (1984) has suggested that they are primarily responsible for the sensation of DOMS. Group IV receptors respond to mechanical, chemical and noxious stimuli (Ebbeling & Clarkson, 1989). It appears that the initial mechanical damage to muscle fibres results in a series of events that cause sensitization of these pain afferents but the exact series of events is unclear.

Stauber *et al.* (1990) have suggested that swelling (an increase in local tissue pressure) is the primary mechanism by which pain afferents are sensitized. They hypothesized that muscle fibre and/or connective tissue disruption leads to the formation of protein fragments and the release of protein-bound ions. The associated increase in osmotic pressure combined with an increase in permeability of muscle capillaries results in fluid influx into damaged myofibrils which causes swelling. However, the evidence for a relationship between oedema and DOMS is contradictory. Bobbert *et al.* (1986) found an increase in limb volume 24, 48 and 72 hours after eccentric calf exercise. They hypothesized that this was due to oedema formation that resulted in soreness. Talag

(1973), however, reported the greatest increases in limb volume of the forearm flexors after eccentric exercise occurred at 72 hours while peak soreness occurred at 48 hours. Studies that have measured intramuscular pressure in sore muscles have also yielded conflicting results (Pyne, 1994b). Finally, in general, anti-inflammatory drugs have not been successful in significantly reducing muscle soreness (Donnelly *et al.*, 1988; Donnelly *et al.*, 1990; Kuipers *et al.*, 1985) which has led Ebbeling & Clarkson (1989) to suggest that either soreness was not related to oedema, or the oedema observed by several investigators was not inflammation in the classic sense. Interestingly, there is evidence from animal experiments that some anti-inflammatory drugs may reduce the extent of exercise-induced damage (Salminen & Kihlstrom, 1987).

Smith (1991) has stated that although substances such as histamine, acetylcholine, bradykinin, potassium and serotonin may stimulate pain afferents, the most likely chemical stimulant may be prostaglandin E_2 (PGE_2). It was suggested that macrophages, present in muscle tissue as a result of an acute inflammatory response to muscle damage, were the primary source of this PGE_2. Smith *et al.* (1993) hypothesized that macrophages were stimulated to produce PGE_2 by elevated intracellular Ca^{2+} levels. Smith (1991) and Smith *et al.* (1993) proposed that contraction or palpation of a muscle caused an increase in intramuscular pressure which provided a mechanical stimulus for the PGE_2-sensitized receptors. Evidence which supported the role of PGE_2 in the generation of muscle soreness was provided by Smith *et al.* (1993) who found that levels of PGE_2 in plasma were significantly related to DOMS. In contrast to the results of Smith *et al.* (1993), Croisier *et al.* (1996) failed to find an increase in PGE_2 after eccentric exercise. The exact role, if any, of PGE_2 in the etiology of DOMS has yet to be established.

(2.2.5) Inflammation and exercise-induced muscle damage.

To date, the precise role of inflammation in EIMD and DOMS has not been clearly defined (Smith, 1991; Pyne, 1994b). It is possible that the inflammatory response may be responsible for initiating, amplifying and/or resolving skeletal muscle injury (MacIntyre *et al.*, 1996). It is clear that the response of the muscle to eccentrically-

induced injury shares many qualitative similarities with processes characteristic of "classical" acute inflammation (Smith, 1991; Pyne, 1994b; MacIntyre *et al.*, 1996; Nosaka & Clarkson, 1996). As described above, pain and loss of function have been consistently observed after eccentric exercise of human muscle in the literature (Smith, 1991). Swelling and inflammatory cell infiltration (mainly neutrophils and macrophages) have been demonstrated on a consistent basis also (Cleak & Eston, 1992; Bobbert *et al.*, 1986; Smith, 1991; Tidball, 1995; Nosaka & Clarkson, 1996; MacIntyre *et al.*, 1996; Round *et al.*, 1987; Faulkner *et al.*, 1993).

However, there are some characteristics of classical acute inflammation that have not been consistently demonstrated after muscular overload. For example, heat and redness have generally not been observed probably due to the deep location of the injury to muscle after eccentric exercise (Smith, 1991; Bobbert *et al.*, 1986). Also, as mentioned previously, it has not been conclusively shown that anti-inflammatory drugs are capable of reducing the extent of EIMD and DOMS (Kuipers *et al.*, 1985; Donnelly *et al.*, 1988; Donnelly *et al.*, 1990; Bourgeois *et al.*, 1999). If classical acute inflammation was a major mechanism involved then one would expect that this class of drugs should have more of a demonstrable effect. In addition, classical acute inflammation typically involves a local and a systemic response, the latter being referred to as the "acute phase response" (Smith *et al.*, 1991; Evans & Cannon, 1991).

The acute phase response exerts both antibacterial and antiviral actions, promotes clearance of damaged tissue and also sets the stage for repair and growth (Evans & Cannon, 1991). It is characterized by activation of complement, increased production of acute phase proteins (such as fibrinogen, haptoglobin, C-reactive protein (CRP), α_1-acid glycoprotein and α_1-antitrypsin) from the liver and a redistribution of trace metals within the body (Evans & Cannon, 1991; Smith & Roberts, 1994). The primary mediators of acute phase protein synthesis are a class of small immunomodulatory polypeptide molecules referred to as cytokines (Evans & Cannon, 1991). This class of molecules includes lymphokines, monokines, chemokines and various growth and necrosis factors (Sigal & Ron, 1994). Cytokines are produced by a wide variety of cells, including immune cells, and are critical for effective signalling between cells of the immune system

(Sigal & Ron, 1994). Interleukin-1 (IL-1), interleukin-6 (IL-6) and tumour necrosis factor (TNF), in particular, have been associated with increased hepatic production of acute phase proteins (Evans & Cannon, 1991; MacIntyre *et al.*, 1996). IL-1, IL-6 and TNF together with IL-2 and interferon-γ (IFN-γ) are generally considered to be the principal mediators of inflammation within the body (Pyne, 1994b).

It appears that the generation of an acute phase response depends to a greater extent on the duration rather than the intensity of the exercise and also on the amount of muscle mass used in the activity (Evans & Cannon, 1991; Nosaka & Clarkson, 1996). The appearance of cytokines and acute phase proteins in the bloodstream and complement activation has been demonstrated after prolonged strenuous whole-body exercise (including downhill running) by several investigators (Evans et al., 1986; Dufaux & Order, 1989; Evans & Cannon, 1991; Northoff & Berg, 1991; Cannon *et al.*, 1991). However, isolated high force eccentric exercise has generally failed to elicit a systemic acute phase response (Sorichter, 1995; Nosaka & Clarkson, 1996). A limitation of the studies that have studied isolated muscles after high-force lengthening contractions is that they have generally failed to monitor levels of cytokines within skeletal muscle itself. It is possible that the amount of damage caused by isolated eccentric exercise is not of a sufficient magnitude to elicit a systemic acute phase response. A further complicating factor when comparing studies in this area may be the use of different cytokine assays with differing sensitivities and specificities (Nosaka & Clarkson, 1996).

Damage to muscle initiates a sequence of cellular responses that varies little, regardless of the nature of the initial insult (Tidball, 1995). These cellular responses appear to be critical for the successful repair of damaged muscle (Tidball, 1995). At least two general cell populations respond to muscle injury: (i) inflammatory cells that remove cellular debris and provide chemical signals for the co-ordination of inflammation and regeneration and (ii) myogenic cells which are involved in the replacement of the damaged muscle (Tidball, 1995). Exactly how inflammation is initiated after injury is a source of debate but Tidball (1995) has suggested a number of potential causes. First, inflammation may be initiated by a "wound hormone" such as muscle cytosolic proteins, basic fibroblast growth factor (bFGF) or platelet-derived growth factor (PDGF). This wound hormone may be capable of activating resident macrophages or fibroblasts which

may then provide the signals necessary to initiate chemotaxis of additional inflammatory cells into damaged muscle. Second, increased extracellular matrix proteolysis may result in the production of proteolytic fragments that are chemoattractants for inflammatory cells. Third, resident macrophages may be directly activated after injury and as a result may produce a number of cytokines that would facilitate progress of muscle inflammation. Examples of such cytokines include transforming growth factor-α (TGF-α), TGF-β, IL-1α, IL-1β and PDGF, all of which are chemoattractants to inflammatory cells. IL-1 in particular has a broad range of inflammatory effects and may induce the expression of many other cytokines (Pyne, 1994b; Tidball, 1995). Finally, it is possible that inflammation may be initiated by activation of the complement system (Tidball, 1995).

The major inflammatory cells that invade damaged muscle are phagocytic polymorphonuclear neutrophils (PMNs) and macrophages (Tidball, 1995). The histological hallmark of acute inflammation is the accumulation of PMNs at the damaged site (MacIntyre *et al.*, 1995). These are the first cells to appear at the site of the injury, within hours of the initial insult (Tidball, 1995; Smith, 1991). They are rapidly recruited in large numbers from the bloodstream by the processes of adhesion to the vascular endothelium, transendothelial migration and chemotaxis to a local inflammatory site (Lloyd & Oppenheim, 1992). Specific adhesive interactions between PMNs and microvascular endothelial cells utilize the CD11/CD18 complex of adhesion molecules on the neutrophil, and endothelial leucocyte adhesion molecules including ICAM-1, ICAM-2 and E-selectin (ELAM-1) (Lloyd & Oppenheim, 1992). Transendothelial migration appears to be an active process and chemoattractant cytokines, such as IL-8, control the movement of PMNs through the extravascular space to the inflammatory site (Lloyd & Oppenheim, 1992).

Once localized, the PMNs ingest and phagocytose particles such as bacteria, tissue fragments and immune complexes bearing immunoglobulin-G (Ig-G) or complement component C3 breakdown products (Lloyd & Oppenheim, 1992; Pyne, 1994a). PMNs are also capable of generating superoxide (O_2^-) free radicals via a "respiratory burst" as they possess the plasma membrane bound nicotinamide adenine

dinucleotide phosphate (NADPH) oxidase enzyme complex (Pyne, 1994a). The capacity of the respiratory burst largely determines the cytotoxic potential of the neutrophil (Pyne, 1994a). As well as playing an important role in the efferent (phagocytosis and degranulation) limb of the immune response, neutrophils are capable of synthesizing and releasing a number of immunoregulatory cytokines and therefore are also important in the afferent limb of the immune response (Lloyd & Oppenheim, 1992; Pyne, 1994a). Hence, neutrophils probably provide additional signals for the chemotaxis of the next wave of inflammatory cells from the circulatory system to the damage site: macrophages.

PMNs are the predominant inflammatory cell at the damage site during the first few hours after injury but after about 12 hours macrophages become the predominant cell type (Tidball, 1995). These cells are probably the single most important cell type in muscle inflammation as, in addition to removing tissue debris by phagocytosis and producing oxygen free radicals, they also release numerous cytokines that influence the regenerative roles of other cells, such as damaged muscle fibre, satellite cells and fibroblasts (Tidball, 1995; MacIntyre *et al.*, 1995). Tidball (1995) has suggested that functionally distinct subclasses of macrophages (particularly those that possess the ED1 and ED2 antigens) play distinct roles in the muscle response to injury. $ED1^+$ macrophages appear to be responsible mostly for phagocytosis in the initial stages after damage whereas $ED2^+$ macrophages, which can secrete numerous cytokines, have been implicated in the later regenerative processes after injury (Tidball, 1995). Tidball (1995) has implicated bFGF, PDGF and IL-1 as the major cytokines involved in the inflammatory and regenerative stages of the muscle response to injury.

(2.2.6) Free radicals and exercise-induced muscle damage.

Free radicals are unstable, highly reactive molecules or fragments of molecules with unpaired electrons in their outer orbitals (Sjödin *et al.*, 1990). Much attention has been paid to oxygen free radicals and their potential role as mediators of skeletal muscle damage and inflammation (Pyne, 1994b; Sjödin *et al.*, 1990; Jenkins, 1988). Oxygen free radicals are produced by a number of exercise-related sources including electron leak within mitochondria (specifically from reduced ubisemiquinone within the electron

transport chain), activation of the xanthine oxidase system in endothelial cells and activation of the NADPH oxidase system in PMNs and macrophages (Pyne, 199b; Sjödin *et al.*, 1990). It has been hypothesized that generation of oxygen free radicals during exercise may initiate lipid peroxidation and membrane damage, eventually leading to cell injury (Pyne, 199b; Sjödin *et al.*, 1990). Membranes that may be damaged within the muscle cell by free radicals include the cell membrane itself, the sarcoplasmic reticular membrane and/or the mitochondrial membrane (Ebbeling & Clarkson, 1989).

The most widely studied oxygen free radicals have been the superoxide anion (O_2^-) and the hydroxyl radical (OH^-), the latter being one of the most reactive free radicals known (Sjödin *et al.*, 1990). It has also been suggested that intermediate nitrogen free radicals, such as peroxynitrite ($ONOO^-$), may be produced if superoxide anion combines with nitric oxide (Pyne, 1994b). Free radical production may also result in the production of further reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2) and hypochlorous acid (HOCl) (Sjödin *et al.*, 1990; Jenkins, 1988).

Under resting conditions, the generation of oxygen free radicals is counterbalanced by a number well developed scavenger and anti-oxidant systems that protect host tissues from oxidative damage (Sjödin *et al.*, 1990; Jenkins, 1988). These systems include the enzymes superoxide dismutase and catalase, the 3-amino acid peptide glutathione and vitamins C and E (Sjödin *et al.*, 1990; Jenkins, 1988). However, during strenuous exercise when oxygen utilization increases markedly, these defence systems may be overwhelmed and oxidative damage may occur to cellular components. Indeed, studies that have shown an increase in lipid peroxidation after exercise have generally used strenuous or exhaustive endurance exercises (Ebbeling & Clarkson, 1989). There is far less evidence supporting a role for free radicals in the initiation or augmentation of injury during high force eccentric exercise of isolated muscles. The only study that could be found that has suggested a significant role for free radicals in the initiation of exercise-induced injury was that of Zerba *et al.* (1990). These investigators found that treatment of mice with superoxide dismutase alleviated evidence of delayed onset histological damage in muscle and also attenuated the reduction of maximum isometric tetanic force seen after a protocol of lengthening contractions.

A number of methodological concerns have been expressed in this area of research (Sjödin *et al.*, 1990; Pyne, 1994b). Most studies have used indirect methods to detect lipid peroxidation, including measurement of malondialdehyde (MDA), thiobarbituric acid reactive substances (TBARS), conjugated dienes, protein carbonyl derivatives, chemiluminescence and expired pentane (Pyne, 1994b; Saxton *et al.*, 1994; Sahlin *et al.*, 1991). In particular, the TBARS technique has been widely used but it is not specific - TBA reacts with MDA as well as with other non-lipid compounds (Sjödin *et al.*, 1990). The only direct method of measuring oxygen free radicals is by electron spin resonance spectroscopy (ESR) but this technique demands very careful tissue handling and is expensive and thus few investigators have used it. A final concern with many studies is that most have investigated levels of free radical markers in the bloodstream (Sahlin *et al.*, 1991; Sjödin *et al.*, 1990). It is likely that the concentration of lipid peroxidation products will be higher in tissue than in plasma and thus it has been suggested that future studies should perform measurements directly in skeletal muscle which would allow for a more accurate assessment of free radical mediated damage (Sahlin *et al.*, 1991; Sjödin *et al.*, 1990).

(2.2.7) The repeated bout, rapid adaptation or training effect

Many studies have demonstrated that physical conditioning, and particularly performance of prior eccentric exercise, causes adaptations to occur in muscle such that the muscle is more resistant to subsequent damage from a similar bout of exercise (Clarkson *et al.*, 1992; Ebbeling & Clarkson, 1989; Ebbeling & Clarkson, 1990; Clarkson & Tremblay, 1988; Nosaka *et al.*, 1991; Mair *et al.*, 1995). It has been shown that this adaptation is initiated very early in the recovery process and probably before the muscle is fully restored (Mair *et al.*, 1995; Ebbeling & Clarkson, 1990). It has also been demonstrated that the length of the adaptation effect varies among the different indicators of muscle damage with the duration of the effect being particularly dramatic for creatine kinase release from muscle. Nosaka *et al.* (1991) found that the repeated bout effect lasted 6 weeks for strength recovery, muscle shortening ability (flexed arm angle) and

soreness, 10 weeks for muscle contractures (relaxed arm angle) and 6 months for the creatine kinase response.

Clarkson & Tremblay (1988) have shown that even a relatively small eccentric insult will produce this adaptation. These investigators demonstrated that performance of a bout of 24 maximal eccentric contractions of the elbow flexors resulted in significantly smaller changes (relative to the control arm which performed no prior exercise) in criterion measures of EIMD when a bout of 70 maximal eccentric contractions was performed two weeks later. This finding, together with the observation that the adaptation effect lasts for a prolonged period of time in the case of CK release (Nosaka *et al.*, 1991) probably contributes, at least in part, to the observation that individuals vary widely in their CK responses to eccentric exercise (Clarkson & Ebbeling, 1988). It is quite possible that those individuals who are "low" CK responders have been "pre-adapted" by prior eccentric exercise. This underlines the importance of using untrained subjects in studies investigating responses of muscle to eccentrically induced damage. It may be difficult to demonstrate significant changes in certain indices of muscle damage (particularly CK release) in subjects who have performed some form of eccentric exercise of the muscle or muscle group concerned during the weeks or months preceding a research study.

The precise location or reason for the training response of muscle is still uncertain and numerous hypotheses have been proposed (Clarkson *et al.*, 1992; McHugh *et al.*, 1999). Alterations in energy metabolism and structural adaptations have been proposed though recent evidence points to the latter as being of more importance (Ebbeling & Clarkson, 1989). A popular theory was the "stress susceptible fibre" hypothesis of Armstrong *et al.* (1983) which suggested that an initial bout of novel exercise resulted in a temporary reduction in a pool of fibres that may be more fragile or more susceptible to damage than other fibres. However, Clarkson & Tremblay (1988) have reasoned that since prior performance of a relatively small bout of eccentric exercise conferred protection for subsequent exercise without itself causing substantial damage, the stress susceptible hypothesis was not tenable. These authors suggested that adaptation was a result of strengthening of the sarcolemma and/or the associated endomysium, a view shared by other investigators (Schwane & Armstrong, 1983). This interpretation,

however, seems inadequate to explain the long-lasting adaptation for CK (Clarkson *et al.*, 1992). These authors argued that with the constant turnover of cellular components, it was unlikely that the strengthened cellular components would remain as such without further stimulus (*ie.* continued eccentric training). It was suggested that altered motor unit recruitment might be involved in the repeated bout effect, since motor skills were known to be "stored" for long periods of time. In a recent review of this area, McHugh *et al.* (1999) concluded that the repeated bout effect occurs through the interaction of various neural, connective tissue and cellular factors that are dependent on the particulars of the eccentric exercise bout and the specific muscle groups involved.

(2.2.8) The role of ^{31}P magnetic resonance spectroscopy and ^{1}H magnetic resonance imaging in the detection of EIMD

The phenomenon of nuclear magnetic resonance (NMR) was discovered in the 1940's and in recent years this technique has become a very valuable one for the non-invasive study of muscle bioenergetics during exercise (Sapega *et al.*, 1987; McCully *et al.*, 1988b). NMR can be used either to determine concentrations of various chemical compounds in human muscle by means of magnetic resonance spectroscopy (MRS) or to produce high quality images using magnetic resonance imaging (MRI). Both MRS and MRI have proved useful in studying healthy and diseased skeletal muscle (McCully *et al.*, 1992).

One of the first studies that used ^{31}P-MRS to monitor human muscle after performance of damaging exercise was that of Aldridge *et al.* (1986). These authors demonstrated that performance of eccentric exercise of the wrist flexor muscles resulted in an increase in inorganic phosphate (P_i) in the resting damaged muscles at 24 and 48 hours post-exercise which coincided with delayed onset muscular soreness. No significant changes in other metabolites or intracellular pH were noted (Aldridge *et al.*, 1986). These results have been supported by several more studies (McCully *et al.*, 1988a; Rodenburg *et al.*, 1994b; Rodenburg *et al.*, 1995). Each of these studies demonstrated an increase in the ratio of P_i/PCr in muscles subjected to lengthening contractions that typically peaked approximately 24 hours post-exercise and remained

elevated for up to 10 days post-exercise (McCully *et al.*, 1988a). Rodenburg *et al.* (1994b) showed that the time course of changes in P_i/PCr differed from that of strength recovery and therefore deduced that the decrease in force generation after eccentric exercise was not solely due to the altered metabolic state within the muscle cell. Concentric (shortening) contractions of muscle were shown not to elicit a delayed increase in P_i/PCr (McCully *et al.*, 1988a).

The precise cause of increased P_i/PCr within eccentrically damaged muscles remains debatable. A number of possible mechanisms have been proposed including an increased activity of ion transport pumps to compensate for "leaky" membranes, alterations in mitochondrial function and an influx of P_i-rich extracellular fluid into damaged muscle cells (McCully *et al.*, 1988a; Aldridge *et al.*, 1986). However, the most widely accepted explanation of raised intracellular P_i following eccentric exercise is an increase in overall resting cell metabolism owing to repair processes such as increased protein synthesis (Rodenburg *et al.*, 1994b; McCully *et al.*, 1988a; McCully & Posner, 1992). Since ^{31}P-MRS is a noninvasive method that reliably and repeatably measures P_i/PCr in skeletal muscle, changes in the ratio of P_i/PCr can be a sensitive indicator of the metabolic status of normal active subjects (McCully *et al.*, 1988b).

Whereas MRS has been typically used to study ^{31}P nuclei in the field of exercise physiology, MRI has been used to study ^{1}H nuclei, or protons (McCully *et al.*, 1992). Protons possess the largest biologically occurring magnetic moment (Allen, 1990). This, combined with the fact that water is the most concentrated molecular species in vivo, results in the production of an intense biological NMR signal (Allen, 1990). This signal can be exploited to produce high quality images with excellent spatial resolution. Compared to MRS, MRI uses larger volume radio-frequency coils and gradient magnetic fields that allow 2-D and even 3-D images to be constructed (McCully *et al.*, 1992). The intensity of the image generated depends on the rate of relaxation of the protons in the sample area. Images of varying contrast can be generated by observing certain different types of nucleus relaxation, specifically T_1 and T_2 relaxation (McCully *et al.*, 1992). T_2-weighted proton images have been particularly useful in detecting differences in the chemical environment of cellular water, though T_1 relaxation has also been investigated. Exercise changes the chemical properties of water molecules in muscle, probably as a

result of an increase in total water content in muscle (particularly extracellular fluid). This results in longer T_1 and T_2 relaxation times and brighter signals within muscle immediately post-exercise (Fisher *et al.*, 1990; Shellock *et al.*, 1991a; Fleckenstein *et al.*, 1988; Takahashi *et al.*, 1994). The magnitude of these immediate post-exercise changes has been shown to be linearly related to exercise intensity (Fisher *et al.*, 1990; Fleckenstein *et al.*, 1988).

A different pattern of change in proton relaxation times has been noted after sports-related muscle injuries, including exercise-induced muscle damage. Fleckenstein *et al.* (1989) and Shellock *et al.* (1991b) were among the first to demonstrate that lengthening contractions of muscle resulted in delayed increases in T_2 relaxation times and signal intensities, which outlasted all other indicators of muscle injury. In fact, in the study of Shellock *et al.* (1991b), MRI showed subclinical abnormalities that lasted as long as 75 days after the disappearance of symptoms in two subjects. The degree of T_2 changes varied considerably between subjects, in both intensity and in the anatomical extent of the injury; a finding consistent with the results of other studies that have used different measurements of muscle injury (Shellock *et al.*, 1991b). The T_2 increase reached a peak between days 3-5 post-exercise for the majority of subjects and was attributed to oedema development within muscle. Shellock *et al.* (1991b) also demonstrated that similar exercise using shortening contractions did not alter T_2 relaxation times and signal intensities.

Delayed increases in muscle T_2 relaxation times and signal intensities similar in magnitude and time course to those found by Fleckenstein *et al.* (1989) and Shellock *et al.* (1991b) have been demonstrated after eccentric exercise in numerous studies in recent years (Nosaka & Clarkson, 1996; Sorichter *et al.*, 1995; Mair *et al.*, 1992; Takahashi *et al.*, 1994; Nurenburg *et al.*, 1992; Rodenburg *et al.*, 1994b). Investigators have consistently attributed early (within one week post-exercise) increases to the accumulation of fluid, or oedema, within muscle. The proposed mechanism(s) by which oedema may develop in muscle after damaging exercise has been discussed in a previous section of this review. As muscle oedema rarely last more than one week but T_2 relaxation changes may last 2-3 months (Shellock *et al.*, 1991b), McCully *et al.* (1992) have speculated that regenerating or recovering muscle fibres may have a different water

chemistry compared with "normal" fibres. Therefore, it is now thought that a quantitative and qualitative change of protons contained in muscle may cause the delayed increase in T_2 seen after EIMD (Takahashi *et al.*, 1994).

Only one study could be found that failed to demonstrate an increase in proton relaxation times within muscle subjected to eccentric exercise (Rodenburg *et al.*, 1995). However, these authors acknowledged that the exercise protocol used to elicit muscle damage may not have been strenuous enough to elicit significant muscle damage. Also, it was not stated if the subjects used were untrained individuals and hence it is possible that their muscles may have been "preadapted" to eccentric exercise.

The sensitivity of MRI for the detection of areas of EIMD has led Nurenburg *et al.* (1992) to suggest that MRI-guided biopsy, as opposed to biopsy guided by DOMS, would provide more accurate information regarding the extent and location of muscle injury and should play an important role in future studies of EIMD. These investigators showed that there was poor correlation between DOMS and CK and the extent of ultrastructural muscle injury but that there was good correlation between signal intensity grades by MRI and the degree of ultrastructural injury. MRI has also been used in several studies to investigate changes in cross sectional area of muscle following a protocol of lengthening contractions (Takahashi *et al.*, 1994; Rodenburg *et al.*, 1994b; Nosaka & Clarkson, 1996). These studies have demonstrated that muscle CSA increases in a delayed manner with a time course similar to the changes in signal intensity and T_2 relaxation in muscle. Presumably these results reflect the effect of oedema development within damaged muscle.

As discussed above, most previous studies that have monitored recovery from EIMD have typically done so using invasive techniques such as blood or muscle tissue sampling (Clarkson & Ebbeling, 1988; Van der Meulen *et al.*, 1991). The most widely used method of assessment of exercise-induced muscle damage has been the measurement of levels of myocellular enzymes within the bloodstream. It is widely acknowledged that this is an indirect method of monitoring muscle damage with a number of significant drawbacks, most notably that levels of plasma myoenzymes do not correlate well with other markers of muscle damage and are not valid indicators of the *extent* of muscle damage (Clarkson & Ebbeling, 1988; Van der Meulen *et al.*, 1991).

MRI/MRS has a number of major advantages over these "traditional" methods of monitoring muscle damage. First, MRI/MRS is non-invasive and, unlike several other forms of imaging/scanning, involves no harmful ionizing radiation. Therefore subjects may be monitored with this technique on numerous occasions without fear of harming the subject. Muscle biopsies are invasive and unpleasant for subjects and it is not reasonable to expect subjects to give numerous muscle biopsy samples over a relatively short time period. Second, unlike muscle biopsy, MRI/MRS can be used to investigate whole muscles. Muscle biopsy only allows sampling of a small amount of muscle tissue at a specific location within an individual muscle. Finally, MRI/MRS allows evidence of muscle damage to be observed within the muscle itself. Direct visualization of signal intensities within muscle by MRI/MRS after eccentric exercise has been found to have less variability and a better correlation than biopsies with the degree of ultrastructural damage within muscle (Nurenburg *et al.*, 1992).

To summarize, the use of MRS and MRI to assess exercise-induced muscle damage is a relatively young field of research. However, results obtained to date have demonstrated that they are promising techniques for investigating the nature, extent and time course of this phenomenon.

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